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UNITED STATES ENVIRONMENTAL PROTECTION AGENCY

WASHINGTON, D.C. 20460

OFFICE OF PREVENTION, PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

DATE: **3/1/04**

SUBJECT: **2,4-D.** Residue data (DERs) supporting the Reregistration Eligibility Decision and use on highbush blueberries, hops, and postharvest use on orange. Case 0073. PC Code 030001. DP Barcodes D235983, D276792, D283959, and D285505. MRIDs 44135201, 44190301, 44190302, 44424801, 44268501, 44577801, 44967401, 45245601, 45462201, 45512701, 45647101, 45665801, and 45672201.

FROM: William J. Hazel, Ph.D., Chemist
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THROUGH: Whang Phang, Ph.D., Branch Senior Scientist
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TO: Mark Seaton, Ph.D.
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and

Joanne Miller
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Attached are a number of technical reviews of residue chemistry data on 2,4-D generated by Task Force II, IR-4, and the California Citrus Quality Council to support reregistraion of 2,4-D as well as to support registration for use on highbush blueberries, hops (PP#2E6352), and orange (postharvest). The regulatory utility of these data have been presented in the Residue Chemistry Chapter of the 2,4-D RED [W. Hazel, 3/1/04, D287660].

RDI: W.Phang: 1/3/04
W.Hazel:CM2:rm.722J:wjh:305-7677:1/3/04

OCT 26 2004

2,4-D Dimethylamine/PC Codes 30019/EDM Industries, Inc.
DACO 7.4.1/OPPTS 860.1500/OECD IIA 6.3.1, 6.3.2, 6.3.3 and IIIA 8.3.1, 8.3.2, 8.3.3
Crop Field Trial - Orange

Primary Evaluator	Dynamac Corporation 1910 Sedwick Rd. Durham, NC 27713 Contract No. 68-W-99-053	Date: 7/01/03
Reviewer	William J. Hazel, Ph.D., Chemist RRB1, HED (7509C)	Date: 3/1/04
Through	Whang Phang, Ph.D., Senior Scientist RRB1, HED (7509C)	

STUDY REPORTS:

45672201 Tieu, H. (2002) Magnitude of Weedaxe (2,4-D) Residue in Citrus: Lab Project Number: R270206. Unpublished study prepared by Primus Labs. 95 p.

EXECUTIVE SUMMARY:

In a single field trial conducted in CA during 2002, 2,4-D dimethylamine (DMA, 0.14 lb ae/gal EC) was applied as a single, directed application at 0.07 lb ae/A to the soil in an orange orchard late in the season. A single control and duplicate treated samples of oranges were harvested 15 days after treatment. Samples were stored at approximately 11 C for 2 days prior to analysis using Method 402 E2 C1 listed in PAM Vol. I. The limit of quantitation for the method was reported to be 0.05 ppm. Besides the results from the sample analyses, details of the exact procedures used by the analytical laboratory and raw data supporting these analyses were not provided. Residues of 2,4-D were <0.05 ppm in/on the 2 samples of treated oranges harvested 15 days post-treatment in the one field trail.

STUDY/WAIVER ACCEPTABILITY/DEFICIENCIES/CLARIFICATIONS:

The regulatory intent of the study sponsor should be provided. If registration of a 2,4-D DMA salt product for use on citrus in the U.S. is the intent, the study is not acceptable as an insufficient number of tests were conducted. Agency guidance requires a minimum of 16 orange field trials, or in cases where expected residues are <LOQ, a minimum of 12 orange field trials. If the registrant intends to support a general use on citrus orchards then a total of 23 field trials are required: 12 on oranges, 5 on lemons, and 6 on grapefruit. In addition, although the method used for analysis was identified, no other information or raw data were provided supporting the results of the analyses. Information on how samples were prepared for analysis and exactly how the method was conducted by the laboratory should be provided along with supporting raw data such as example calculations, chromatograms, standard curves, and data spread sheets.

The acceptability of this study for regulatory purposes is discussed in the Residue Chemistry Chapter of the 2,4-D RED [W. Hazel, 3/1/04, D287660].

COMPLIANCE:

Signed and dated GLP, quality assurance, and data confidentiality statements were provided. No deviations from regulatory requirements were noted that would impact the study results or their interpretation.

A. BACKGROUND INFORMATION

2,4-Dichlorophenoxyacetic acid (2,4-D) is a List A pesticide active ingredient classified as an herbicide, a plant growth regulator, and a fungicide. It is, however, mainly used as a selective postemergence herbicide for the control of certain weed species on a variety of food/feed sites including field, fruit, and vegetable crops. In addition to 2,4-D acid, there are eight salts and esters of 2,4-D, each with an assigned PC Code number, which are presently registered as active ingredients in herbicide end-use products (EPs). Uses of 2,4-D are currently being supported mainly by the Industry Task Force II on 2,4-D Research.

EDM Industries, Inc., which is not a member of the Industry Task Force, has submitted field trial data from a single orange field trial presumably to support the use of a specific 2,4-D DMA end-use product in citrus orchards. This formulation is a 0.14 lb ae/gal EC (EPA Reg. No. 68292-2; label dated 11/15/2002,Weedaxe® Herbicide) that is not currently registered for control of weeds in citrus orchards, but is registered for controlling weeds in pome fruit, stone fruit, and tree nut orchards at a maximum rate 0.07 lb ae/A.

TABLE A.1. Nomenclature of Test Compound

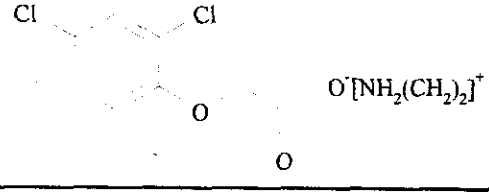
Compound	
Common name	2,4-D DMA
Company experimental names	None
IUPAC name	dimethylamine (2,4-dichlorophenoxy) acetate
CAS name	(2,4-dichlorophenoxy) acetic acid, dimethylamine salt
CAS #	2008-39-1
End-use products/EP	0.14 lb ae/gal EC; EPA Reg. No. 68292-2; Weedaxe® Herbicide

TABLE A.2. Physicochemical Properties of 2,4-D DMA		
Parameter	Value	Reference (MRID)
Melting point/range	118-120 C	42829901
pH	6.8-9	not available
Density	1.23 g/cm^3	not available
Water solubility (25°C)	pH 5 321 g/L pH 7 729 g/L pH 9 664 g/L	not available
Solvent solubility (20°C)	acetonitrile 10.2 g/L methanol >500 g/L hexane 35.9 g/L 1-octanol 53.7 g/L toluene 1.65 g/L	not available
Vapor pressure at 25°C	<1.3 x 10^{-5} Pa	not available
Dissociation constant (pK_a)	3	not available
Octanol/water partition coefficient Log(K_{ow})	-0.83 at pH 7	not available
UV/visible absorption spectrum (λmax, nm)	not available	not available

B. EXPERIMENTAL DESIGN

B.1. Study Site Information

Information on cultural practices (cultivation and maintenance chemicals), soil characterization (Table B.1.1), and climatic data (temperature and rainfall) were provided. The oranges were grown using standard cultural practices and no unusual weather conditions were noted during the field trial. Supplemental irrigation was used as needed.

TABLE B.1.1 Soil Characterization.				
Study Location (City, State), Year	Soil characteristics			
	Type	%OM	pH	CEC (meq/ 100 g)
Porterville, CA, 2002	Loam	NR [1]	NR	NR

[1] NR = Not reported.

TABLE B.1.2. Study Use Pattern on Valencia Oranges.							
Location (City, State) Year	Application						
	Formulation	Timing [1]	Rate (lb a.e./A)	No. of Appl.	Method [2]	Volume (gal/A)	Tank Mix Adjuvants
Porterville, CA, 2002	0.14 lb ae/gal EC	late season	0.07	1	directed	30	None

[1] The application was made approximately 15 days prior to normal maturity.

[2] Application was made using ground equipment as a directed ground application direct to the orchard floor.

TABLE B.1.3. Trial Numbers and Geographical Locations [1]

NAFTA Growing Region [1]	Total Orange Field Trials		
	Submitted	Requested	
		Canada	US [2]
1	-	NA	--
2	--	NA	--
3	--	NA	11 (8)
4	--	NA	--
5	--	NA	--
6	--	NA	1 (2)
7	--	NA	--
8	--	NA	--
9	--	NA	--
10	1	NA	4 (3)
11	-	NA	1
12	-	NA	1
Total	1	NA	16 (12)

[1] Regions 13-21 and 1A, 5A, 5B, and 7A were not included as the use is for the US only.
[2] Number of trials in parentheses is for 25% reduction in number of trials due to nonquantifiable residues.
NA = not applicable.

B.2. Analytical Methodology

Residues of 2,4-D in/on whole oranges were reportedly analyzed using Method 402 E2 C1 listed in Pesticide Analytical Methods (PAM), Vol. I. A copy of the method as printed in PAM Vol. I was included in the submission; however, no other details regarding the analyses were given.

The submission did not contain any information from the analytical laboratory on the exact procedures used to prepare samples and analyze 2,4-D residues. In addition, no raw data were provided supporting the analyses such as sample calculations, example chromatograms, and standard curves. The only information provided from the analytical laboratory consisted of residue reporting sheets, which listed the residue values for each sample.

C. RESULTS AND DISCUSSION

The number and geographic representation of the field trial data are not adequate. Only 1 of the required 16 field trials was conducted. Even assuming that residues from all orange field trials would be <LOQ, a minimum of 12 field trials would be required to support this use on oranges. In addition, if the registrant intends to support a general use on citrus orchards then a total of 23 field trial would be required: 12 on oranges, 5 on lemons, and 6 on grapefruit.

Common cultural practices were used to maintain the orchard, and the weather conditions and the maintenance chemicals used in the study did not have a notable impact on the residue data.

A single control and duplicate treated samples of mature oranges were harvested 15 days after application and placed on frozen gel packs. The samples were transferred to EMD personnel on the day of collection and then shipped by overnight courier (temperature unspecified) to the analytical laboratory (Primus Labs, Santa Maria, CA). Storage temperatures at the analytical laboratory were not provided. However, the samples were analyzed within 2 days of harvest (Table C.1); therefore, supporting storage stability data are not required for this study.

Residues of 2,4-D in/on orange samples were determined using Method 402 E2 C1 listed in PAM Vol. I; however, details of the exact procedures used by the analytical laboratory and raw data supporting these analyses were not provided. Therefore, the adequacy of the method as conducted by the performing laboratory could not be evaluated. Only residue values for each sample were reported. A footnote on the residues reporting sheets noted that the recovery of 2,4-D was 67% from a control sample spiked at 0.15 ppm. Apparent residues in/on the control orange sample were reported to be <LOQ.

In one orange field trial conducted in CA during 2001, 2,4-D DMA (0.14 lb ae/gal EC) was applied as a single, late-season, directed application to the orchard floor at 0.07 lb ae/A. Residues of 2,4-D were <0.05 ppm in/on 2 samples of treated oranges harvested 15 days post-treatment (Table C.2).

TABLE C.1 Summary of Storage Conditions

Matrix	Storage Temp. (C)	Actual Storage Duration from Harvest to Analysis (days)	Limit of Demonstrated Storage Stability (days) [1]
Whole oranges	11	2	NA

[1] NA = not applicable; storage stability are not required given the short duration of storage.

TABLE C.2 Residue Data on Oranges from Field Trials with 2,4-D DMA.

Location (City, State), Year	EPA Region	Variety	Total Rate (lb ae/A)	PHI (days)	2,4-D Residues (ppm) [1]
Porterville, CA, 2002	10	Cutter Valencia Orange	0.07	15	<0.05, <0.05

[1] The reported LOQ is 0.05 ppm.

D. CONCLUSION

The submitted residue data on oranges are not adequate. Only 1 of the required 16 orange field trials was conducted, and insufficient information was provided pertaining to the analyses conducted by the analytical laboratory.

E. REFERENCES

None

2,4-D Dimethylamine/PC Codes 30019/EDM Industries, Inc.
DACO 7.4.1/OPPTS 860.1500 and 1520/OECD IIA 6.3.1, 6.3.2, 6.3.3 and IIIA 8.3.1, 8.3.2, 8.3.3
Crop Field Trial and Processing Study - Grape

Primary Evaluator	Dynamac Corporation 1910 Sedwick Rd. Durham, NC 27713 Contract No. 68-W-99-053	Date: 6/30/03
Reviewer	William J. Hazel, Ph.D., Chemist RRB1, HED (7509C)	Date: 3/1/04
Through	Whang Phang, Ph.D., Senior Scientist RRB1, HED (7509C)	

STUDY REPORTS:

45665801 Tieu, H. (2001) Magnitude of Weedaxe (2,4-D) Residues in Grapes: Lab Project Number: ERS21075: CA01: 21-075. Unpublished study prepared by Primus Labs. 110 p.

EXECUTIVE SUMMARY:

In a single field trial conducted in CA during 2001, 2,4-D dimethylamine (DMA, 0.14 lb ae/gal EC) was applied as a single, directed application at 0.07 lb ae/A to the soil in a grape vineyard at the time of fruit softening. A single control and duplicate treated samples of mature grapes were harvested 15 days after treatment, and bulk samples were also harvested and pressed to obtain juice samples. Samples were stored at approximately -20 C for 3 days prior to analysis using Method 402 E2 C1 listed in PAM Vol. I. The limit of quantitation for the method was reported to be 0.05 ppm. Besides the results from the sample analyses, details of the exact procedures used by the analytical laboratory and raw data supporting these analyses were not provided.

Residues of 2,4-D were <0.05 ppm in/on 2 samples of treated grapes harvested 15 days post-treatment from one field trail and were <0.05 ppm in 2 samples of juice derived from the treated grapes.

STUDY/WAIVER ACCEPTABILITY/DEFICIENCIES/CLARIFICATIONS:

The study is not acceptable as an insufficient number of tests were conducted. Agency guidance requires a minimum of 12 grape field trials, or in cases where expected residues are <LOQ, a minimum of 9 grape field trials. Even if the use is restricted only to CA, a minimum of 5-6 field trials would be required. In addition, although the method used for analysis was identified, no other information or raw data were provided supporting the results of the analyses. Information on how exactly the method was conducted by the laboratory should be provided along with supporting raw data, such as example calculations, chromatograms, standard curves, and data spread sheets. Also, if the registrant intended to provide data on grape processed commodities,

the study was not conducted at a high enough application rate, and samples of raisins were not collected. Agency guidance requires use of exaggerated application rates for processing studies where residues in the RAC are expected to be <LOQ following treatment at the maximum labeled rate. In the case of grapes, which have a theoretical concentration factor of 4.7 for raisins, the grape processing study should use an application at 5x the maximum labeled rate.

The acceptability of this study for regulatory purposes is discussed in the Residue Chemistry Chapter of the 2,4-D RED [W. Hazel, 3/1/04, D287660].

COMPLIANCE:

Signed and dated GLP, quality assurance, and data confidentiality statements were provided. No deviations from regulatory requirements were noted that would impact the study results or their interpretation.

A. BACKGROUND INFORMATION

2,4-Dichlorophenoxyacetic acid (2,4-D) is a List A pesticide active ingredient classified as an herbicide, a plant growth regulator, and a fungicide. It is, however, mainly used as a selective postemergence herbicide for the control of certain weed species on a variety of food/feed sites including field, fruit, and vegetable crops. In addition to 2,4-D acid, there are eight salts and esters of 2,4-D, each with an assigned PC Code number, which are presently registered as active ingredients in herbicide end-use products (EPs). Uses of 2,4-D are currently being supported mainly by the Industry Task Force II on 2,4-D Research.

EDM Industries, Inc., which is not a member of the Industry Task Force, has submitted field trial data from a single grape field trial to support the use of a specific 2,4-D DMA end-use product in grape vineyards. This formulation is a 0.14 lb ae/gal EC (EPA Reg. No. 68292-2; Weedaxe® Herbicide) that was previously registered for use in grape vineyards as a single, directed application to weeds at 0.035 lb ae/A with a pre-harvest interval (PHI) of 40 days. According to a Federal Register Notice dated 9/27/2000 (*FR Notice Vol. 65, No. 188, p 58703-58704*), the registrant requested cancellation of the use on grapes for this product, and the latest copy of the accepted label for EPA Reg. No. 68292-2 (dated 11/15/2002) indicates that the use on grape has been dropped form the label.

EDM appears to be supporting use of a late-season (15-day PHI) directed ground application of 2,4-D DMA to grape vineyards at a maximum of 0.07 lb ae/A. For comparison, the Industry Task Force II is supporting the use in CA of a single, directed ground application of 2,4-D (amine, salts, and acid) in grape vineyards around the time of bloom at a maximum rate of 1.36 lb ae/A, with a 100 day PHI.

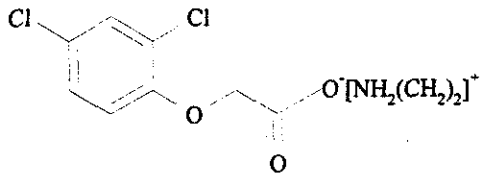
TABLE A.1. Nomenclature of Test Compound	
Compound	
Common name	2,4-D DMA
Company experimental names	None
IUPAC name	dimethylamine (2,4-dichlorophenoxy) acetate
CAS name	(2,4-dichlorophenoxy) acetic acid, dimethylamine salt
CAS #	2008-39-1
End-use products/EP	0.14 lb ae/gal EC; EPA Reg. No. 68292-2; Weedaxe® Herbicide

TABLE A.2. Physicochemical Properties of 2,4-D DMA		
Parameter	Value	Reference (MRID)
Melting point/range	118-120 C	42829901
pH	6.8-9	not available
Density	1.23 g/cm^3	not available
Water solubility (25°C)	pH 5 321 g/L pH 7 729 g/L pH 9 664 g/L	not available
Solvent solubility (20°C)	acetonitrile 10.2 g/L methanol >500 g/L hexane 35.9 g/L 1-octanol 53.7 g/L toluene 1.65 g/L	not available
Vapor pressure at 25°C	<1.3 x 10^{-5} Pa	not available
Dissociation constant (pK$_a$)	3	not available
Octanol/water partition coefficient Log(K$_{OW}$)	-0.83 at pH 7	not available
UV/visible absorption spectrum (λmax, nm)	not available	not available

B. EXPERIMENTAL DESIGN

B.1. Study Site Information

Information on cultural practices (cultivation and maintenance chemicals), soil characterization (Table B.1.1), and climatic data (temperature and rainfall) were provided. The grapes were grown using standard cultural practices and no unusual weather conditions were noted during the field trial. Supplemental irrigation was used as needed.

TABLE B.1.1 Soil Characterization.

Study Location (City, State), Year	Soil characteristics			
	Type	%OM	pH	CEC (meq/ 100 g)
Fresno, CA, 2001	Sandy Loam	NR [1]	NR	NR

[1] NR = Not reported.

TABLE B.1.2. Study Use Pattern on Grapes.

Location (City, State) Year	Application						
	Formulation	Timing [1]	Rate (lb a.e./A)	No. of Appl!	Method [2]	Volume (gal/A)	Tank Mix Adjuvants
Fresno, CA, 2001	0.14 lb ae/gal EC	late season, berry softening	0.07	1	directed	30	None

[1] The application was made approximately 15 days prior to normal maturity.
[2] Application was made using ground equipment as a directed ground application to the vineyard floor.

TABLE B.1.3. Trial Numbers and Geographical Locations [1]

NAFTA Growing Region [1]	Total Grape Field Trials		
	Submitted	Requested	
		Canada	US [2]
1	-	NA	2
2	--	NA	--
3	--	NA	--
4	--	NA	--
5	--	NA	--
6	--	NA	--
7	--	NA	--
8	--	NA	--
9	--	NA	--
10	1	NA	8 (5)
11	-	NA	1
12	-	NA	1
Total	1	NA	12 (9)

[1] Regions 13-21 and 1A, 5A, 5B, and 7A were not included as the use is for the US only.
[2] Number of trials in parentheses is for 25% reduction in number of trials due to non-quantifiable residues.
NA = not applicable.

B.2. Analytical Methodology

Residues of 2,4-D in/on whole grapes and juice were reportedly analyzed using Method 402 E2 C1 listed in Pesticide Analytical Methods (PAM), Vol. I. A copy of the method as printed in

PAM Vol. I was included in the submission; however, no other details regarding the analyses were given.

The submission did not contain any information from the analytical laboratory on the exact procedures used for analysis or on the equipment used for determination of 2,4-D residues. In addition, no raw data were provided supporting the analyses, such as sample calculations, example chromatograms, and standard curves. The only information provided from the analytical laboratory consisted of residue reporting sheets, which listed the residue values for each sample.

C. RESULTS AND DISCUSSION

The number and geographic representation of the field trial data are not adequate. Only 1 of the required 12 field trials was conducted. Even assuming that residues from all grape trials would be <LOQ and that the use was restricted to CA, a minimum of 5 field trials would be required to support this use on grapes.

Common cultural practices were used to maintain the vineyard, and the weather conditions and the maintenance chemicals used in the study did not have a notable impact on the residue data.

A single control and duplicate treated samples of mature grapes were harvested 15 days after application. In addition, bulk samples of grapes were pressed immediately after sampling to obtain a single control and two treated samples of juice. The samples were transported on ice to EMD on the day of collection and placed in frozen storage (-20 C). Samples were transferred in a frozen state to the analytical laboratory (Primus Labs, Santa Maria, CA) and were analyzed within two days. As the frozen grape and juice samples were analyzed within 3 days of harvest (Table C.1), supporting storage stability data are not required for this study.

Residues of 2,4-D in/on grape and juice samples were determined using Method 402 E2 C1 listed in PAM Vol. I; however, details of the exact procedures used by the analytical laboratory and raw data supporting these analyses were not provided. Therefore, the adequacy of the method as conducted by the performing laboratory could not be evaluated. Only residue values for each sample were reported. A footnote on the residue reporting sheets noted that the recovery of 2,4-D was 85% from a sample spiked at 0.5 ppm; however, there was no indication of whether this was a grape or juice sample. Apparent residues in/on the control grape and juice samples were reported to be <LOQ.

In one field trial conducted in CA during 2001, 2,4-D DMA (0.14 lb ae/gal EC) was applied as a single, late-season, directed application to the vineyard floor at 0.07 lb ae/A. Residues of 2,4-D were <0.05 ppm in/on 2 samples of treated grapes harvested 15 days post-treatment (Table C.2) and were also <0.05 ppm in 2 samples of juice derived from the treated grapes.

TABLE C.1 Summary of Storage Conditions			
Matrix	Storage Temp. (C)	Actual Storage Duration from Harvest to Analysis (days)	Limit of Demonstrated Storage Stability (days) [1]
Whole grapes and juice	-20	3	NA

[1] NA = not applicable; frozen storage stability are not required given the short duration of storage.

TABLE C.2 Residue Data on Grapes and Grape Juice from Field Trials with 2,4-D DMA.						
Location (City, State), Year	EPA Region	Variety	Matrix	Total Rate (lb ae/A)	PHI (days)	2,4-D Residues (ppm) [1]
Fresno, CA, 2001	10	Thompson Seedless	Whole grapes	0.07	15	<0.05, <0.05
			Juice	0.07	15	<0.05, <0.05

[1] The LOQ is 0.05 ppm.

D. CONCLUSION

The submitted residue data on grapes and grape juice are not adequate. Only 1 of the required 12 grape field trials was conducted, and insufficient information was provided pertaining to the analyses conducted by the analytical laboratory. In addition, the application rate was too low to adequately assess the potential for concentration of residues in grape processed fractions, and no raisin samples were collected. Agency guidance requires use of exaggerated application rates for processing studies where residues in the RAC are expected to be <LOQ following treatment at the maximum labeled rate. In the case of grapes, which have a theoretical concentration factor of 4.7 for raisins, a grape processing study should use an application at 5x the maximum labeled rate.

E. REFERENCES

None

2,4-D/PC Codes 30001 and 30034/IR-4 Project
DACO 7.4.1/OPPTS 860.1500/OECD IIA 6.3.1, 6.3.2, 6.3.3 and IIIA 8.3.1, 8.3.2, 8.3.3
Processed Food and Feed - Grape

Primary Evaluator	Dynamac Corporation 1910 Sedwick Rd. Durham, NC 27713 Contract No. 68-W-99-053	Date: 6/27/03
Reviewer	William J. Hazel, Ph.D., Chemist RRB1, HED (7509C)	Date: 3/1/04
Through	Whang Phang, Ph.D., Senior Scientist RRB1, HED (7509C)	

STUDY REPORT:

45647101 Kunkel, D. (1996) 2,4-D: Magnitude of the Residue on Grape: Lab Project Number: 04298: ENC-2/93: 4298.94-CA70. Unpublished study prepared by Rutgers University. 242 p. (note this submission is identical to MRID 43947901, which was never reviewed)

EXECUTIVE SUMMARY:

In two tests conducted in CA during 1994, a multiple active ingredient (MAI) formulation of 2,4-D was applied as a single, directed ground application to vineyards at 1.425 lb ae/A. The application was made in spring at early fruit set, prior to substantial vine growth. The MAI formulation contained 9.5% of 2,4-D acid and 48.65% of 2,4-D triethylamine salt for a total of 42.86% 2,4-D acid equivalents (3.8 lb ae/gal SC/L). Although uses for the triethylamine salt of 2,4-D are no longer being supported, data from this form of 2,4-D can be considered representative of other amine salt forms of 2,4-D, such as the dimethylamine salt.

Bulk samples of treated grapes were collected from each site at 101 or 104 days post-treatment. Samples from one site were immediately pressed for grape juice, and samples from the other site were field-dried for raisins. Samples of whole grapes, juice, and raisins were stored frozen for ~15 months prior to analysis, an interval supported by available stability data.

Grape matrices were analyzed for residues of 2,4-D using GC/ECD method EN-CAS Method No. ENC-2/93, with minor modifications. The method was validated and found to be adequate for data collection. For this method, residues are extracted from grape matrices with 0.5 M KOH in ethanol:H_2O (1:1, v/v), filtered, and refluxed for 1 hour in 0.4 M HCl. Hydrolyzed residues are then cleaned-up using a C_{18} solid phase extraction column, concentrated to dryness and then derivatized to the methyl ester with diazomethane. The derivatized residues are then dissolved in 25% toluene in hexane, cleaned-up using an Alumina column eluted with 25% toluene in hexane, and analyzed by GC/ECD. The LOQ for 2,4-D is 0.05 ppm in/on grapes, grape juice, and raisins. The LOD was not reported.

Following a single, early-season, directed application of 2,4-D MAI (2,4-D acid and triethylamine salt, 3.8 lb ae/gal SC/L) to the vineyard floor at 1.425 lb ai/A, residues of 2,4-D were <0.05 ppm in/on all 4 samples of grapes and in 2 samples each of juice and raisins derived from the treated grapes. As residues were <LOQ, processing factors could not be determined.

STUDY/WAIVER ACCEPTABILITY/DEFICIENCIES/CLARIFICATIONS:

Under the conditions and parameters used in the study, the grape processing study is classified as scientifically unacceptable. In addition, the study can not be upgraded. Although residues were <LOQ in all grape samples used for processing and in the resulting grape juice and raisin samples, the field trail was conducted at only 1x by maximum labeled use rate.

The acceptability of this study for regulatory purposes is also addressed in the Residue Chemistry Chapter of the 2,4-D RED [W. Hazel, 3/1/04, D287660].

COMPLIANCE:

Signed and dated GLP, quality assurance, and data confidentiality statements were provided. No deviations from regulatory requirements were noted that would impact the study results or their interpretation.

A. BACKGROUND INFORMATION

2,4-Dichlorophenoxyacetic acid (2,4-D) is a List A pesticide active ingredient classified as an herbicide, a plant growth regulator, and a fungicide. It is, however, mainly used as a selective postemergence herbicide for the control of certain weed species on a variety of food/feed sites including field, fruit, and vegetable crops. In addition to the parent acid, there are eight salts and esters of 2,4-D, each with an assigned PC Code number, which are presently registered as active ingredients in herbicide end-use products (EPs).

To support the use of 2,4-D in grape vineyards, the registrant has submitted a data reflecting the potential for 2,4-D residues occurring in processed fractions derived from grapes grown in vineyards treated with a MAI formulation of 2,4-D (acid and triethylamine salt, 3.8 lb ae/gal SC/L) as a single directed ground application at 1.425 lb ae/A, prior to vines reaching the ground in spring.

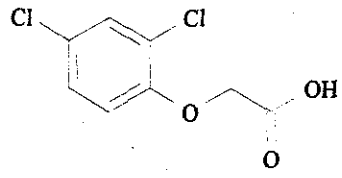
TABLE A.1. Nomenclature of 2,4-D	
Compound	
Common name	2,4-D
Company experimental names	N/A
IUPAC name	2,4-dichlorophenoxyacetic acid
CAS name	(2,4-dichlorophenoxy)acetic acid
CAS #	94–75-7
End-use products/EP	3.8 lb ae/gal SC/L; MAI containing acid and triethylamine salt of 2,4-D

TABLE A.2. Physicochemical Properties		
Parameter	Value	Reference)
Melting point/range (C)	138-141	2,4-D RED
pH	Not available	
Density (25°C)	1.416	
Water solubility (20°C)	569 mg/L	
Solvent solubility (g/100 g at 25 °C)	acetone 85.0 benzene 1.07 diethyl ether 220 ethanol 130.0 isopropanol 31.6 toluene 0.067 xylene 0.58	
Vapor pressure (25°C)	1.4×10^{-7} mm Hg	
Dissociation constant (pK_a) at 25°C	3	
Octanol/water partition coefficient Log(K_{OW})	2.83	
UV/visible absorption spectrum (λmax, nm)	Not available	

B. EXPERIMENTAL DESIGN

B.1. Application and Crop Information

TABLE B.1. Study Use Pattern on Grapes.

Location (City, State) Year	Application						
	Formulation [1]	Timing [2]	Rate (lb a.e./A)	No. of Appl.	Method [3]	Volume (gal/A)	Tank Mix Adjuvants
Fresno, CA, 1994	3.8 lb ae/gal SC/L	early fruit set	1.425	1	directed	48	None
Five Points, CA, 1994						49	None

[1] The formulation is a MAI containing 9.5% of 2,4-D acid and 48.65% of 2,4-D triethylamine salt for a total of 42.86% 2,4-D acid equivalents (3.8 lb ae/gal).

[2] The application was made in the spring prior to extensive vine growth.

[3] Application was made using ground equipment as a directed ground application to the vineyard floor.

B.2. Processing Procedures

Bulk samples from both test sites were processed immediately after harvest. At one of the sites, grapes were pressed in a juicer to yield juice and pomace samples, which were immediately frozen. At the other site, whole bunches of grapes were field-dried on paper lined trays for 20 days to yield raisins, which were frozen immediately after collection. Samples of whole grapes, juice and raisins were stored frozen (<-20 C) at the field sites for 3-52 days prior to shipment by freezer truck to the analytical laboratory (IR-4 Western Region Laboratory, Davis, CA).

B.3. Analytical Methodology

The GC/ECD method EN-CAS Method No. ENC-2/93, with minor modifications, was used for determining residues of 2,4-D in/on grapes and grape processed fractions. This method was previously validated and found to be adequate for data collection in/on various plant commodities (D. Miller, 1/24/96, CBRS No. 14004, DP Barcode D205346). A brief description of the method follows.

Residues are extracted into 0.5 M KOH in ethanol:H_2O (1:1, v/v) and filtered. The resulting extract is refluxed for 1 hour in 0.4 M HCl. Hydrolyzed residues are then cleaned-up using a C_{18} solid phase extraction column by rinsing with water and hexane, and then eluting residues with hexane:ethyl acetate (1:1, v/v). Residues are concentrated to dryness and then derivatized to the methyl ester with diazomethane. The derivatized residues are then dissolved in 25% toluene in hexane and cleaned-up using an Alumina column eluted with 25% toluene in hexane. Methylated residues are determined by GC/ECD.

The analytical laboratory validated the above GC/ECD method using control samples of grapes, juice, and raisins each fortified with 2,4-D at 0.05 and 0.50. The LOQ for 2,4-D is 0.05 ppm in/on each commodity. The LOD was not reported.

C. RESULTS AND DISCUSSION

In two tests conducted in CA during 1994, a MAI formulation of 2,4-D (3.8 lb ae/gal SC/L) was applied as a single, directed ground application to vineyards at 1.425 lb ae/A. The application was made at early fruit set, prior to substantial vine growth. Bulk samples of treated grapes were collected from each site at 101 or 104 days post-treatment. Samples from one site were immediately pressed for grape juice, and samples from the other site were field-dried for raisins. Samples of grapes, juice, and raisins were frozen and shipped by freezer truck to the analytical laboratory, where the samples were stored at -20 C. The total frozen storage interval for the grapes and processed fractions was ~15 months (Table C.2.1).

To support this storage interval, three control samples each of grapes, juice, and raisins were fortified with 2,4-D at 0.5 ppm and stored at -20 C at the analytical laboratory, along with the processing samples. The stored samples of each matrix were analyzed after ~15 months of frozen storage along with two freshly fortified samples and a control sample. No initial zero-day samples were analyzed; however, the average corrected recovery of 2,4-D from the stored samples was 95% for grapes after 467 days, 103% for juice after 466 days, and 96% for raisins after 414 days (Table C.2.2). These data are adequate to support the storage intervals from this processing study.

Grape matrices were analyzed for residues of 2,4-D using GC/ECD method EN-CAS Method No. ENC-2/93, with minor modifications. The method was validated by the analytical laboratory using control samples of grapes, juice, and raisins fortified at 0.05 or 0.5 ppm and analyzed concurrently with the treated samples. Concurrent method recoveries averaged 79, 71, and 92% from grape, juice, and raisin samples, respectively (Table C.1). Apparent residues of 2,4-D were <0.05 ppm in/on all control samples. The validated LOQ for 2,4-D is 0.05 ppm in/on grape matrices. The LOD was not reported. Adequate sample calculations and chromatograms were provided.

Following a single early-season directed application of 2,4-D MAI (2,4-D acid and triethylamine salt, 3.8 lb ae/gal SC/L) to the vineyard floor at 1.425 lb ai/A, residues of 2,4-D were <0.05 ppm in/on all 4 samples of grapes and in 2 samples each of juice and raisins derived from the treated grapes. As residues were <LOQ, processing factors could not be determined.

TABLE C.1 Summary of Concurrent Recoveries of 2,4-D from Grapes and Grape Processed Fractions.

Matrix	Analyte	Spike level (ppm)	Sample size (n)	Recoveries (%)	Mean ± std dev
Whole fruit	2,4-D	0.05, 0.5	8	64-93 (1)	79 ± 10
Juice		0.05, 0.5	8	64-78 (4)	71 ± 6
Raisins		0.05, 0.5	8	81-107	92 ± 8

[1] The number of recoveries outside the acceptable 70-120% range is in parentheses.

TABLE C.2.1 Summary of Freezer Storage Conditions.

Apple Matrix	Storage Temp. (°C)	Actual Storage Duration (days)	Limit of Demonstrated Storage Stability (days) [1]
Whole fruit	-20	456-457	467
Juice		463	466
Raisins		438	414

[1] A frozen storage stability study was conducted concurrently with the processing study.

TABLE C.2.2 Stability of 2,4-D in Grape Matrices Following Storage at -20°C.

Commodity	Analyte	Spike level (mg/kg)	Storage interval (days)	Recovered residues (mg/kg)		Corrected % recovery [1]
				Fresh fort.	Stored Fort.	
Grapes	2,4-D	0.5	467	0.395, 0.408 (0.402) [2]	0.392, 0.357, 0.391 (0.380) [2]	94.5
Juice	2,4-D	0.5	466	0.450, 0.431 (0.441)	0.470, 0.448, 0.448 (0.455)	103.2
Raisins	2,4-D	0.5	414	0.318, 0.320 (0.319)	0.302, 0.314, 0.304 (0.307)	96.2

[1] Corrected for average concurrent recovery.
[2] Average of two (fresh) or three (stored) samples is listed in parentheses.

TABLE C.3. Residue Data from Grape Processing Study with 2,4-D MAI. [1]

RAC	Processed Commodity	Total Rate [2] (lb ae/A)	PTI (days)	2,4-D Residues (ppm) [3]	Processing Factor
Grape	whole grapes	1.425	101	<0.05, <0.05	NA
	Juice			<0.05, <0.05	NA
Grape	Whole grapes	1.425	104	<0.05, <0.05	NA
	Raisins			<0.05, <0.05	NA

[1] The formulation used in this study was a MAI containing 9.5% of 2,4-D acid and 48.65% of 2,4-D triethylamine salt for a total of 42.86% 2,4-D acid equivalents (3.8 lb ae/gal).
[2] The 1x rate for grapes is 1.36 lb ae/A.
[3] The LOQ is 0.05 ppm.

NA = not applicable

D. CONCLUSION

The grape processing study is not adequate. Although residues were <LOQ in all grape samples used for processing and in the resulting grape juice and raisin samples, the field trail was conducted at only ~1x the maximum labeled rate. Agency guidance requires use of exaggerated application rates for processing studies where residues in the RAC are expected to be <LOQ following treatment at the maximum labeled rate. In the case of grapes, which have a theoretical concentration factor of 4.7 for raisins, the grape processing study should use an application at 5x the maximum labeled rate.

E. REFERENCES

CB No.: 14004
DP Barcode: D205346
Subject: 2,4-D. Enforcement Analytical Method for Plants. GDLN 171-4(c).
From: D. Miller
To: J. Coombs
Dated: 1/26/96
MRID(s): 43289301

Primary Evaluator	Dynamac Corporation 1910 Sedwick Rd. Durham, NC 27713 Contract No. 68-W-99-053	Date: 6/27/03
Reviewer	William J. Hazel, Ph.D., Chemist RRB1, HED (7509C)	Date: 3/1/04
Through	Whang Phang, Ph.D., Senior Scientist RRB1, HED (7509C)	

STUDY REPORTS:

45647101 Kunkel, D. (1996) 2,4-D: Magnitude of the Residue on Grape: Lab Project Number: 04298: ENC-2/93: 4298.94-CA70. Unpublished study prepared by Rutgers University. 242 p. (note this submission is identical to MRID 43947901, which was never reviewed)

EXECUTIVE SUMMARY:

In two tests conducted in CA during 1994, a multiple active ingredient (MAI) formulation of 2,4-D was applied as a single, broadcast application to the soil in grape vineyards at 1.425 lb ae/A. The application was made in spring at early fruit set, prior to substantial vine growth. The MAI formulation contained 9.5% of 2,4-D acid and 48.65% of 2,4-D triethylamine salt for a total of 42.86% 2,4-D acid equivalents (3.8 lb ae/gal SC/L). Although uses for the triethylamine salt of 2,4-D are no longer being supported, data from this form of 2,4-D can be considered representative of other amine salt forms of 2,4-D, such as the dimethylamine salt.

A single control and duplicate treated samples of mature grapes were collected from each test at 101 or 104 days after treatment. Samples were stored at -20 C for 15 months prior to analysis, an interval that is supported by the concurrent storage stability data.

Residues of 2,4-D in/on grapes were determined using GC/ECD method EN-CAS Method No. ENC-2/93, with minor modifications. The method was validated and found to be adequate for data collection. For this method, residues are extracted from grapes with 0.5 M KOH in ethanol:H_2O (1:1, v/v), filtered, and refluxed for 1 hour in 0.4 M HCl. Hydrolyzed residues are then cleaned-up using a C_{18} solid phase extraction column, concentrated to dryness and then derivatized to the methyl ester with diazomethane. The derivatized residues are then dissolved in 25% toluene in hexane, cleaned-up using an Alumina column eluted with 25% toluene in hexane, and analyzed by GC/ECD. The LOQ for 2,4-D is 0.05 ppm in/on grapes. The LOD was not reported.

Following a single, early season, broadcast application of a MAI formulation of 2,4-D (acid plus amine salt, SC/L) at 1.425 lb ae/A to the ground of vineyards, residues of 2,4-D were <0.05 ppm in/on 4 samples of grapes harvested ~100 days posttreatment.

STUDY/WAIVER ACCEPTABILITY/DEFICIENCIES/CLARIFICATIONS:

Although the residue data from the 2 tests conducted in CA are scientifically adequate, the overall study is not acceptable as an insufficient number of tests were conducted. Agency guidance requires a minimum of 12 grape field trials, or in cases where expected residues are <LOQ, a minimum of 9 grape field trials. Even if the use is restricted only to CA, a minimum of 5-6 field trials would be required. However, the residue data in this submission will be considered along with other grape residue data in determining the acceptability of this study for regulatory purposes in the Residue Chemistry Chapter of the 2,4-D RED [W. Hazel, 3/1/04, D287660].

COMPLIANCE:

Signed and dated GLP, quality assurance, and data confidentiality statements were provided. No deviations from regulatory requirements were noted that would impact the study results or their interpretation.

A. BACKGROUND INFORMATION

2,4-Dichlorophenoxyacetic acid (2,4-D) is a List A pesticide active ingredient classified as an herbicide, a plant growth regulator, and a fungicide. It is, however, mainly used as a selective postemergence herbicide for the control of certain weed species on a variety of food/feed sites including field, fruit, and vegetable crops. In addition to 2,4-D acid, there are eight salts and esters of 2,4-D, each with an assigned PC Code number, which are presently registered as active ingredients in herbicide end-use products (EPs).

To support the use of 2,4-D in grape vineyards, IR-4 has submitted residue data reflecting the use of 2,4-D as a single directed ground application to weeds in grape vineyards at 1.425 lb ae/A, prior to vines reaching the ground in spring. The formulation used in this study is an multiple active ingredient formulation containing 9.5% of 2,4-D acid and 48.65% of 2,4-D triethylamine salt, for a total of 42.86% 2,4-D acid equivalents (3.8 lb ae/gal). Although the triethylamine salt of 2,4-D is no longer being supported for use, related amine salt formulations, such as the dimethylamine form of 2,4-D, are still being supported. Therefore, these data can be used to support the general class of 2,4-D amine salts.

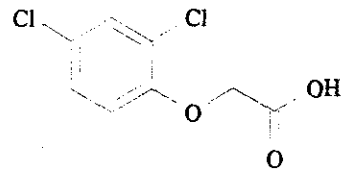
TABLE A.1. Nomenclature of 2,4-D	
Compound	
Common name	2,4-D
Company experimental names	N/A
IUPAC name	2,4-dichlorophenoxyacetic acid
CAS name	(2,4-dichlorophenoxy)acetic acid
CAS #	94–75-7
End-use products/EP	3.8 lb ae/gal SC/L: MAI containing acid and triethylamine salt of 2,4-D

TABLE A.2. Physicochemical Properties			
Parameter	Value		Reference)
Melting point/range (C)	138-141		2,4-D RED
pH	Not available		
Density (25°C)	1.416		
Water solubility (20°C)	569 mg/L		
Solvent solubility (g/100 g at 25 °C)	acetone 85.0 diethyl ether 220 isopropanol 31.6 xylene 0.58	benzene 1.07 ethanol 130.0 toluene 0.067	
Vapor pressure (25°C)	1.4×10^{-7} mm Hg		
Dissociation constant (pK_a) at 25°C	3		
Octanol/water partition coefficient Log(K_{ow})	2.83		
UV/visible absorption spectrum (λmax, nm)	Not available		

B. EXPERIMENTAL DESIGN

B.1. Study Site Information

Information on cultural practices (cultivation and maintenance chemicals), soil characterization (Table B.1.1), and climatic data (temperature and rainfall) were provided. The grapes were grown using standard cultural practices and no unusual weather conditions were noted during any of the trials. Supplemental irrigation was used as needed.

TABLE B.1.1 Soil Characterization.

Study Location (City, State), Year	Soil characteristics			
	Type	%OM	pH	CEC (meq/ 100 g)
Fresno, CA, 1994	Sandy Loam	<1%	7.0	NR [1]
Five Points, CA, 1994	Clay Loam	<0.5	7.8	NR

[1] NR = Not reported.

TABLE B.1.2. Study Use Pattern on Grapes.

Location (City, State) Year	Application						
	Formulation [1]	Timing [2]	Rate (lb a.e./A)	No. of Appl.	Method [3]	Volume (gal/A)	Tank Mix Adjuvants
Fresno, CA, 1994	3.8 lb ae/gal SC/L	early fruit set	1.425	1	directed	48	None
Five Points, CA, 1994						49	None

[1] The formulation is a MAI containing 9.5% of 2,4-D acid and 48.65% of 2,4-D triethylamine salt for a total of 42.86% 2,4-D acid equivalents (3.8 lb ae/gal).

[2] The application was made in the spring prior to extensive vine growth.

[3] Application was made using ground equipment as a directed ground application to the vineyard floor.

TABLE B.1.3. Trial Numbers and Geographical Locations [1]

NAFTA Growing Region [1]	Total Grape Field Trials		
	Submitted	Requested	
		Canada	US [2]
1	-	NA	2
2	--	NA	--
3	--	NA	--
4	--	NA	--
5	--	NA	--
6	--	NA	--
7	--	NA	--
8	--	NA	--
9	--	NA	--
10	2	NA	8 (5)
11	-	NA	1
12	-	NA	1
Total	2	NA	12 (9)

[1] Regions 13-21 and 1A, 5A, 5B, and 7A were not included as the use is for the US only.

[2] Number of trials in parentheses is for 25% reduction in number of trials due to non-quantifiable residues.

NA = not applicable.

B.2. Analytical Methodology

The GC/ECD method EN-CAS Method No. ENC-2/93, with minor modifications, was used for determining residues of 2,4-D in/on grapes. This method was previously validated and found to be adequate for data collection in/on various plant commodities (D. Miller, 1/24/96, CBRS No. 14004, DP Barcode D205346). A brief description of the method follows.

Residues are extracted into 0.5 M KOH in ethanol:H_2O (1:1, v/v) and filtered. The resulting extract is refluxed for 1 hour in 0.4 M HCl. Hydrolyzed residues are then cleaned-up using a C_{18} solid phase extraction column by rinsing with water and hexane, and then eluting residues with hexane:ethyl acetate (1:1, v/v). Residues are concentrated to dryness and then derivatized to the methyl ester with diazomethane. The derivatized residues are then dissolved in 25% toluene in hexane and cleaned-up using an Alumina column eluted with 25% toluene in hexane. Methylated residues are determined by GC/ECD.

The analytical laboratory validated the above GC/ECD method using control samples of grapes fortified with 2,4-D at 0.05 and 0.50 ppm. The LOQ for 2,4-D is 0.05 ppm in/on grapes. The LOD was not reported.

C. RESULTS AND DISCUSSION

The number and geographic representation of the field trial data are not adequate. Only 2 of the required 12 field trials were conducted. Even assuming that residues from all grape trials would be <LOQ and that the use was restricted to CA, a minimum of 5 field trials would be required to support this use on grapes.

Common cultural practices were used to maintain the vineyards, and the weather conditions and the maintenance chemicals used in the study did not have a notable impact on the residue data.

A single control and duplicate treated samples of mature grapes were collected from the two tests at 101 or 104 days after application. The reported PHI is 100 days for grapes. Samples were stored (<-19 C) at the field sites for 3-52 days prior to shipment by freezer truck to the analytical laboratory (IR-4 Western Region Laboratory, Univ. of CA, Davis, CA), where samples were stored at <-20 C until analysis. The total frozen storage interval was ~460 days for grape samples (Table C.2.1).

To support this storage interval, three control samples of grapes were fortified with 2,4-D at 0.5 ppm and stored at -20 C at the analytical laboratory. The stored samples were analyzed after 467 days of frozen storage along with two freshly fortified samples and a control sample. No initial zero-day sample was analyzed; however, the average corrected recovery of 2,4-D from the stored samples was 95% after 467 days (Table C.2.2). These data are adequate to support the storage intervals from this field trail.

The GC/ECD method (EN-CAS Method No. ENC-2/93) is adequate for determining residues of 2,4-D in/on grapes. Concurrent method recoveries averaged 82 ± 15% from control samples fortified at 0.05 ppm and 77 ± 10% from control samples fortified at 0.5 ppm (Table C.1). Apparent residues of 2,4-D were <0.05 ppm in/on all control grape samples. The validated LOQ for 2,4-D is 0.05 ppm in/on grapes. The LOD was not reported. Adequate sample calculations and chromatograms were provided.

In two field trials conducted in CA during 1994, a MAI of 2,4-D (3.8 lb ae/gal SC/L) was applied as a single directed application to the vineyard floor at 1.425 lb ae/A. The application was made at early fruit set, prior to vines reaching the ground. Residues of 2,4-D were <0.050 ppm in/on all 4 samples of grapes harvested ~100 days post-treatment (Table C.3).

TABLE C.1 Summary of Concurrent Recoveries of 2,4-D from Grapes.

Matrix	Analyte	Spike level (ppm)	Sample size (n)	Recoveries (%)	Mean ± std dev
Grape	2,4-D	0.05	3	70, 84, 93	82 ± 12
		0.5	5	64-88 (1)	77± 10

[1] The number of recoveries outside the acceptable 70-120% range is in parentheses.

TABLE C.2.1 Summary of Storage Conditions

Matrix (RAC)	Storage Temp. (C)	Actual Storage Duration from Harvest to Analysis (days)	Limit of Demonstrated Storage Stability (days) [1]
Grape Fruit	-20	456-457	467

[1] A storage stability study was conducted by the analytical laboratory concurrently with the field trials.

TABLE C.2.2 Stability of 2,4-D in Grapes Following Storage at -20°C.

Commodity	Analyte	Spike level (mg/kg)	Storage interval (days)	Recovered residues (mg/kg)		Corrected % recovery [1]
				Fresh fort.	Stored Fort.	
Grapes	2,4-D	0.5	467	0.395, 0.408 (0.402) [2]	0.392, 0.357, 0.391 (0.380) [2]	94.5

[1] Corrected for average concurrent recovery.
[2] Average of three samples is listed in parentheses.

TABLE C.3 Residue Data on Grapes from Field Trials with 2,4-D (MAI) [1].					
Location (City, State), Year	EPA Region	Variety	Total Rate (lb ae/A)	PHI (days)	2,4-D Residues (ppm) [2]
Fresno, CA, 1994	10	Thompson Seedless	1.425	104	<0.05, <0.05
Five Points, CA, 1994	10	Thompson Seedless	1.425	101	<0.05, <0.05

[1] The formulation used in this study was a MAI containing 9.5% of 2,4-D acid and 48.65% of 2,4-D triethylamine salt for a total of 42.86% 2,4-D acid equivalents (3.8 lb ae/gal).

[2] The LOQ is 0.05 ppm.

D. CONCLUSION

Although the residue data from the two grape field trials conducted in CA are adequate, the number and distribution of grape field trials were not adequate. Only 2 of the required 12 field trials were conducted.

E. REFERENCES

CBRS No.: 14004
DP Barcode: D205346
Subject: Enforcement Analytical Method for Plants.
From: D. Miller
To: J. Coombs
Date: 1/24/96
MRID(s): 43289301

Template Version March 2003

2,4-D DMA/PC Code: 030019/IR-4
DACO 7.4.1/OPPTS 860.1500/OECD IIA 6.3.1, 6.3.2, 6.3.3 and IIIA 8.3.1, 8.3.2, 8.3.3
Crop Field Trial - Hops

Primary Evaluator	Dynamac Corporation, Contract No. 68-W-99-053	Date: 5/23/03
Reviewer	William J. Hazel, Ph.D., Chemist RRB1, HED (7509C)	Date: 3/1/04
Through	Whang Phang, Ph.D., Senior Scientist RRB1, HED (7509C)	

STUDY REPORT:

45512701 Arsenovic, M. (2001) 2,4-D: Magnitude of the Residue on Hops. Lab Project Number: A5024.99-CAR22. Unpublished study prepared by IR-4 Project. 124 p.

EXECUTIVE SUMMARY:

In a total of 3 field trials conducted in ID, OR, and WA during 1999, the dimethylamine salt (DMA) of 2,4-D (1.38 lb ae/gal SC/L) was applied as three directed applications to the middle of the rows between hops plants at 27- to 33-day retreatment intervals from the vegetative stage through cone development. Applications were made at 0.49-0.52 lb ae/A, for a total of 1.49-1.53 lb ae/A/season. Duplicate mature hops samples were collected and dried 28-30 days after the last application.

Hops cone samples were stored frozen for a maximum of 117 days prior to analysis. This storage interval is supported by the available stability data, which indicate that 2,4-D residues are stable in frozen hops for at least 119 days.

Residues of 2,4-D in/on hops were determined by an GC/ECD method (EN-CAS Method No. ENC-2/93), which was validated and found to be adequate for data collection. For this method, residues are extracted from hops into 0.5 M KOH in ethanol:H_2O (EtOH, 1:1, v/v), filtered, and refluxed for 1 hour in 0.4 M HCl. Hydrolyzed residues are then cleaned-up using a C_{18} solid phase extraction (SPE) column, concentrated to dryness and then derivatized to the methyl ester with diazomethane. The derivatized residues are then partitioned into 25% toluene in hexane, cleaned-up using an Alumina column eluted with 25% toluene in hexane, and analyzed by GC/ECD. The LOQ for 2,4-D is 0.05 ppm in/on hops. The LOD was not reported.

Following three applications of 2,4-D DMA (SC/L) totaling 1.49-1.53 lb ae/A, residues of 2,4-D were <0.050-0.053 ppm in/on 6 samples of 6 dried hop cones harvested 28-30 days post-treatment.

STUDY/WAIVER ACCEPTABILITY/DEFICIENCIES/CLARIFICATIONS:

Under the conditions and parameters used in the study, the hops field trial residue data are classified as scientifically acceptable. The acceptability of this study for regulatory purposes is addressed in the Residue Chemistry Chapter of the 2,4-D RED [W. Hazel, 3/1/04, D287660].

COMPLIANCE:

Signed and dated GLP, quality assurance, and data confidentiality statements were provided. No deviations from regulatory requirements were noted that would impact the study results or their interpretation.

A. BACKGROUND INFORMATION

2,4-dichlorophenoxy acetic acid (2,4-D) is a List A pesticide active ingredient classified as an herbicide, a plant growth regulator, and a fungicide. It is, however, mainly used as a selective postemergence herbicide for the control of certain weed species on a variety of food/feed sites including field, fruit, and vegetable crops. In addition to the parent acid, there are eight salts and esters of 2,4-D, each with an assigned PC Code number, which are presently registered as active ingredients in herbicide end-use products (EPs). To support the use of 2,4-D dimethylamine (DMA) on hops, IR-4 has submitted a petition (PP#2E6352) proposing the use of 2,4-D DMA (1.38 lb/gal SC/L) as directed foliar application to the row middles at 0.5 lb ae/A with up to 3 applications per season. In conjunction with this use, IR-4 is proposing a tolerance of 0.1 ppm for residues of 2,4-D in/on hops.

TABLE A.1. Nomenclature of Test Compound

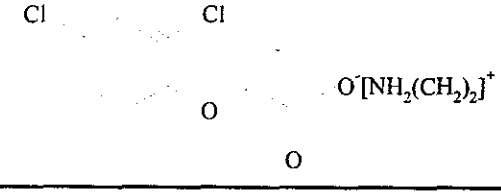
Compound	
Common name	2,4-D DMA
Company experimental names	None
IUPAC name	dimethylamine (2,4-dichlorophenoxy) acetate
CAS name	(2,4-dichlorophenoxy) acetic acid, dimethylamine salt
CAS #	2008-39-1
End-use products/EP	3.8 lb ae/gal SC

TABLE A.2. Physicochemical Properties of 2,4-D DMA		
Parameter	Value	Reference (MRID)
Melting point/range	118-120 C	42829901
pH	6.8-9	not available
Density	1.23 g/cm^3	not available
Water solubility (25°C)	pH 5 321 g/L pH 7 729 g/L pH 9 664 g/L	not available
Solvent solubility (20°C)	acetonitrile 10.2 g/L methanol >500 g/L hexane 35.9 g/L 1-octanol 53.7 g/L toluene 1.65 g/L	not available
Vapor pressure at 25°C	<1.3 x 10^{-5} Pa	not available
Dissociation constant (pK_a)	3	not available
Octanol/water partition coefficient Log(K_{ow})	-0.83 at pH 7	not available
UV/visible absorption spectrum (λmax, nm)	not available	not available

B. EXPERIMENTAL DESIGN

B.1. Study Site Information

Temperatures and rainfall data were collected at each site, and were within average historical values for the residue study period. Rainfall was supplemented with irrigation as needed.

TABLE B.1.1 Soil Characterization.	
Study Location (City, State), Year	Soil Type
Prosser, WA, 1999	Sandy Loam
Hubbard, OR, 1999	Clay Loam
Parma, ID, 1999	Not Provided

TABLE B.1.2. Study Use Pattern on Hops.

Location (City, State) Year	Application [1]							
	Timing [2]	Formulation	Single Rate [3] (lb a.e./A)	RTI [4] (days)	No. of Appl.	Method [5]	Volume (gal/A)	Total Rate [3] (lb a.e./A)
Prosser, WA, 1999	postemergence	1.38 lb ae/gal SC/L	0.49-0.50	27-28	3	directed	17-26	1.49
Hubbard, OR, 1999	postemergence	1.38 lb ae/gal SC/L	0.50-0.52	28-33	3	directed	20-21	1.53
Parma, ID, 1999	postemergence	1.38 lb ae/gal SC/L	0.50-0.51	28-32	3	directed	26	1.52

1 No adjuvants were included in the tank mix for any trial.
2 Applications were made to plants at the vegetative stage (1st), pre-bloom or early flowering (2nd), and cone stage (3rd).
3 Rates were expressed as lb ai/A; however, it appears that the petitioner was referring to the rate in terms of acid equivalents (ae).
4 RTI = Retreatment Interval
5 All applications were made using ground equipment and were directed to weeds growing between the rows.

TABLE B.1.3. Trial Numbers and Geographical Locations

NAFTA Growing Region [1]	Total Hops Trials		
	Submitted	Requested [2]	
		Canada	US
1	--	NA	--
2	--	NA	--
3	--	NA	--
4	--	NA	--
5	--	NA	--
6	--	NA	--
7	--	NA	--
8	--	NA	--
9	--	NA	--
10	--	NA	--
11	2	NA	3
12	1	NA	--
Total	3	NA	3

[1] Regions 13-21 and 1A, 5A, 5B, and 7A were not included as the proposed use is for the US only.

[2] The requested NAFTA growing region number for hops field trials is not specified as the number of trials is not >3.

NA = not applicable.

B.2: Analytical Methodology

The GC/ECD method (EN-CAS Method No. ENC-2/93), for determining residues of 2,4-D in/on plants, was previously validated and found to be adequate for data collection in/on various plant commodities (D. Miller, 1/24/96, CBRS No. 14004, DP Barcode D205346). A brief description of the method follows.

Residues are extracted into 0.5 M KOH in ethanol:H_2O (EtOH, 1:1, v/v) and filtered. The resulting extract is refluxed for 1 hour in 0.4 M HCl. Hydrolyzed residues are then cleaned-up using a C_{18} solid phase extraction column by rinsing with water and hexane, and then eluting with hexane:ethyl acetate (EtOAc, 1:1, v/v). Residues are concentrated to dryness and then derivatized to the methyl ester with diazomethane. The derivatized residues are then partitioned into 25% toluene in hexane and cleaned-up using an Alumina column eluted with 25% toluene in hexane. Methylated residues are determined by GC/ECD.

The analytical laboratory validated the above GC/ECD method using control samples of hops fortified with 2,4-D at 0.05-0.50 ppm. The LOQ for 2,4-D is 0.05 ppm in/on hops. The LOD was not reported.

C. RESULTS AND DISCUSSION

The number of crop field trials and geographic representation of the residue data on hops is adequate according to the latest EPA Guidance.

Duplicate treated and control samples of mature hops were collected from each test 28-30 days after the last application, the cones were placed in a dryer for 2-23 hours. The dried cones (RAC) were placed in frozen storage within 1 hour of removal from the dryer. Samples were stored (<-9 C) at the field sites for 14-27 days prior to shipment by freezer truck to the analytical laboratory (IR-4 Western Region Leader Laboratory, Davis, CA), where samples were stored at <-15 C until analysis. The total frozen (-9 C) storage intervals were 104-117 days for hops samples (Table C.2.1). These storage intervals are supported by the available stability data (Table C.2.2), which indicate that 2,4-D residues are stable in frozen hops for at least 119 days. Data are also available indicating that 2,4-D is stable in a variety of frozen plant commodities for at least 12 months (D. Miller, 3/19/96, CBRS No. 16425, DP Barcode D220451).

The GC/ECD method (EN-CAS Method No. ENC-2/93) for determining residues of 2,4-D in/on plants was validated by the analytical laboratory using control samples of hops and was found to be adequate for data collection. In the hops field trial analyses, the concurrent method recoveries were 62-89% (71 ± 6%) from 11 hops control samples fortified with 2,4-D at 0.05-0.5 ppm (Table C.1). Apparent residues of 2,4-D were <0.05 ppm in/on all control hops samples. The validated LOQ for 2,4-D is 0.05 ppm in/on hops. The LOD was not reported. Adequate sample calculations and chromatograms were provided.

In a total of 3 field trials conducted in ID, OR, and WA during 1999, 2,4-D DMA (1.38 lb ae/gal SC/L) was applied as three directed applications at 0.49-0.52 lb ae/A/application, for a total of 1.49-1.53 lb ae/A/season. The spray was directed to the row middles between hops plants at ~1 month intervals from the vegetative stage through cone development. Duplicate mature hop samples were collected and dried 28-30 days after the last application. Residues of 2,4-D were <0.050-0.053 ppm in/on 6 samples of hop cones harvested 28-30 days post-treatment (Table C.3).

Common cultural practices were used to maintain plants, and the weather conditions and the maintenance chemicals and fertilizer used in the study did not have a notable impact on the residue data.

TABLE C.1 Concurrent Recovery Results from Hops Field Trials for GC/ECD Method (ENC-2/93.

Hops Matrix	Spiking Level (mg/kg)	Sample size	2,4-D	
			Recoveries (%)	Mean Recovery ± SD
Dried Cones (RAC)	0.05, 0.5	11	62-89 (4) [1]	71 ± 6

[1] The number of recoveries outside the acceptable 70-120% range is in parentheses.

In conjunction with the magnitude of the residue studies, IR-4 submitted data on the stability of 2,4-D residues in hops. Three control samples of hop cones were fortified with 2,4-D at 0.5 ppm and held in frozen storage (<-15 C) at the analytical laboratory for 119 days. All storage stability samples were analyzed using the ENC-2/93 method.

The submitted storage stability data are adequate and indicate that residues of 2,4-D are stable at <-15 C for at least 119 days in hop cones.

TABLE C.2.1 Summary of Freezer Storage Conditions

Hops Matrix	Storage Temp. (°C)	Actual Storage Duration (days)	Limit of Demonstrated Storage Stability (days) [1]
Dried Cones	> -15	104-117	119

[1] The submitted stability data for 2,4-D residues in hops matrices stored frozen for 119 days are adequate: additional storage stability data on various commodities indicate 2,4-D residues are stable in frozen storage for at least 12 months (D. Miller, 3/19/96, CBRS No. 16425, DP Barcode D220451).

TABLE C.2.2 Stability of 2,4-D in Dried Hops Following Storage at -20°C.

Commodity	Analyte	Spike level (mg/kg)	Storage interval (days)	Recovered residues (mg/kg)		Corrected % recovery [1]
				Fresh fort.	Stored Fort.	
Dried Hops	2,4-D	0.5	119	0.345, 0.360, 0.310 (0.338) [2]	0.376, 0.389, 0.297 (0.354) [2]	105

[1] Corrected for average concurrent recovery.
[2] Average of three samples is listed in parentheses.

TABLE C.3. Residue Data on Dried Hops from Field Trials with 2,4-D.

Location (City, State, Year)	EPA Region	Hops Variety	Total Rate (lbs ae/A)	PHI (days)	2,4-D Residues (ppm) [1]
Prosser, WA, 1999	11	Nugget	1.49	28	<0.050, <0.050
Hubbard, OR, 1999	12	Nugget	1.53	29	<0.050, <0.050
Parma, ID, 1999	11	Nugget	1.52	30	0.052, 0.053

[1] The LOQ is 0.05 ppm.

TABLE C.4. Summary of Residue Data for Hops from Crop Field Trials with 2,4-D DMA.

Hops Matrix	Total Rate (lb ae/A)	PHI (days)	No of samples	Residue Levels (ppm)				
				Min.	Max.	HAFT [1]	Mean	Std. Dev.
Dried Cones	1.49-1.53	28-30 [2]	11	<0.050 [3]	0.053	0.053	0.034	0.014

[1] HAFT = Highest Average Field Trial.
[2] The proposed PHI is 30 days.
[3] The LOQ is 0.05 ppm in/on hops. For samples having residues <LOQ, ½ the LOQ was used for calculating the average residues.

D. CONCLUSION

The hops field trial data are adequate and reflect the use of 2,4-D DMA (SC/L) at a maximum seasonal application rate of 1.5 lb ae/A, which is 1x the proposed use rate for hops.

E. REFERENCES

CBRS No.: 14004
DP Barcode: D205346
Subject: Enforcement Analytical Method for Plants.
From: D. Miller
To: J. Coombs
Date: 1/24/96
MRID(s): 43289301

CBRS No.: 16425
DP Barcode: D220451
Subject: 2,4-D. (030001) Storage Stability Study on Various Raw and Processed Agricultural Commodities. GDLN 171(e).
From: D. Miller
To: J. Coombs
Dated: 3/19/96
MRID(s): 43809901

2,4-D Isopropyl Ester/PC Code: 030066/California Citrus Quality Council
DACO 7.4.1/OPPTS 860.1500/OECD IIA 6.3.1, 6.3.2, 6.3.3 and IIIA 8.3.1, 8.3.2, 8.3.3
Crop Field Trial - Citrus fruits (postharvest)

Primary Evaluator	Dynamac Corporation 1910 Sedwick Rd. Durham, NC 27713 Contract No. 68-W-99-053	Date: 12/10/02
Reviewer	William J. Hazel, Ph.D., Chemist RRB1, HED (7509C)	Date: 3/1/04
Through	Whang Phang, Ph.D., Senior Scientist RRB1, HED (7509C)	

STUDY REPORT:

45462201 Johnson, G., Strickland, M. (2001) Magnitude of Residues in/on Citrus Fruit After Post Harvest Treatments with (2,4-Dichlorophenoxy)acetic Acid Isopropyl Ester: Lab Project Numbers: 101-014; 6578-108. Unpublished study prepared by California Citrus Quality Council. 176 p.

EXECUTIVE SUMMARY:

In six post-harvest trail runs conducted in CA during 2001, the isopropyl ester of 2,4-dichlorophenoxy acetic acid (2,4-D IPE, ~3.2 lb/gal EC) was applied as a single, post-harvest, dilute aqueous spray to oranges (6 tests) and lemons (4 tests) at a concentration of 534-681 ppm, followed by treatment with a waxing solution. The fruit were treated using standard commercial post-harvest application procedures. Samples were stored frozen for ≤12 days prior to analysis using an adequate GC/MSD method (Method HWI 6578-101B) with an LOQ of 0.05 ppm.

Immediately following the application, residues of 2,4-D were 0.158-0.242 ppm in/on 12 orange samples and 0.326-0.604 ppm in/on 8 lemon samples. Average 2,4-D residues were 0.200 ppm in/on oranges and 0.394 ppm in/on lemons. Residues in/on all control samples were <0.05 ppm.

The study authors noted that citrus fruits can be treated with 2,4-D IPE either as a dilute spray prior to waxing or as a water-wax emulsion containing 2,4-D IPE. Although detailed data were not provided, preliminary data from trials conducted prior to writing the study protocol indicated that 2,4-D residues were slightly higher following aqueous spray application (mean = 0.26 ppm) than following an application in wax (mean = 0.16 ppm). Therefore, extensive tests were conducted only on the aqueous spray.

STUDY/WAIVER ACCEPTABILITY/DEFICIENCIES/CLARIFICATIONS:

Under the conditions and parameters used in the study, the post-harvest treatment data on citrus (lemons and oranges) are classified as scientifically acceptable and reflect the use of 2,4-D IPE (EC) at a maximum concentration of 500 ppm. The acceptability of this study for regulatory purposes is addressed in the Residue Chemistry Chapter of the 2,4-D RED [W. Hazel, 3/1/04, D287660].

COMPLIANCE:

Signed and dated GLP, quality assurance, and data confidentiality statements were provided. No deviations from regulatory requirements were noted that would impact the study results or their interpretation.

A. BACKGROUND INFORMATION

The 2,4-D IPE is used as a plant growth regulator on citrus as a pre-harvest treatment to prevent leaf and fruit drop and as a post-harvest treatment to inhibit abscission of buttons on fruits. In the United States, 2,4-D IPE is currently registered for preharvest application to citrus fruits only in AZ and CA and for post-harvest treatment of only lemons. These uses are being supported by the California Citrus Quality Council (CCQC). The current submission contains only data on the post-harvest use of 2,4-D IPE on citrus fruits; data supporting the preharvest use have been previously reviewed (DP Barcode D221853, D. Miller, 7/8/96).

Although the post-harvest use of 2,4-D IPE is restricted to lemons in the U.S., the current submission also contains residue data on the post-harvest treatment of navel oranges to support the Codex MRL for the post-harvest use of 2,4-D IPE on citrus fruits, as this use is permitted in other countries. In addition to the residue data, the CCQC provided a copy of a 2,4-D IPE label (EC) from Uruguay along with a translation. This label is similar to the post-harvest treatment allowed in the U.S., except that other citrus fruits may be treated in addition to lemons. 2,4-D IPE (EC) may be applied post-harvest to citrus fruits either in a wax solution or as a dilute aqueous flush or spray at a concentration of 500 ppm.

A tolerance of 5 ppm has been established for residues of 2,4-D in/on citrus fruits resulting from the preharvest and post-harvest uses of 2,4-D IPE [40 CFR §180.142 (a)(1)].

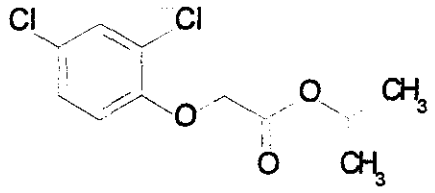
TABLE A.1. Test Compound Nomenclature	
Compound	
Common name	2,4-D IPE
Company experimental names	na
IUPAC name	(2,4-dichlorophenoxy)acetate, isopropyl ester
CAS name	(2,4-dichlorophenoxy) acetic acid, isopropyl ester
CAS #	94-11-1
End-use products/EP	~3.2 lb/gal EC (HIVOL-44 Plant Growth Regulator; EPA Reg. No. 64864-31)

TABLE A.2. Physicochemical Properties	
Parameter	Value
Melting point/range	Liquid at room temperature
pH	NA
Density (25 C)	1.25 g/cm^3
Water solubility	86.7 ppb
Solvent solubility (20°C)	fully miscible in dichloromethane, hexane, isopropanol, and toluene
Vapor pressure at 25°C	3.98 x 10^{-6} mm Hg
Dissociation constant (pK$_a$)	NA
Octanol/water partition coefficient Log(K$_{ow}$)	4.2 at 25 C
UV/visible absorption	NA

NA = not available

B. EXPERIMENTAL DESIGN

B.1. Study Site Information

Navel oranges and Lisbon lemons were purchased commercially and stored for 7 days at ~2 C prior to a postharvest treatment with 2,4-D IPE. None of the fruit received a preharvest treatment with 2,4-D. The postharvest application was conducted at the Sunkist Research Station, Ontario, CA on 1/23/01 using the application equipment on an experimental packing house line that simulates commercial practices.

A total of six trials were run using separate lots of fruit and different treatment solutions (Table B.1). Both lemons and oranges (50 fruits each) were treated together in the first four tests and only oranges were used in the last two tests. Prior to treatment, all fruit were run through a

foamer washer containing a 10% solution of a commercial foamer wash; fruit were then rinsed with water and dried. Separate 5 gallon solutions of 2,4-D IPE were prepared for each test at a target concentration of 500 ppm. Duplicate aliquots of each treatment solution were collected to determine the actual concentration of 2,4-D IPE in each solution.

For each treatment, the fruit were run through the simulated packing line. Fruits were initially sprayed with a dilute aqueous solution of 2,4-D IPE and then run through plastic brushes that were also coated with the treatment solution. The contact time between the fruit and 2,4-D IPE solution was ~22 seconds. After treatment, the fruit were run through foam brushes, where the fruit were partially dried, and then onto wax brushes where a commercial storage wax (Sunshine 625 Lemon Storage Wax) was applied using a solution of 12.5% storage wax in water. Finally, fruit were conveyed through a forced-air dryer (~52 C) to dry the wax. Samples of 20 fruits were collected immediately, subdivided into two 10 fruit subsamples, and placed in frozen storage until shipment the following day by overnight courier on dry ice to the analytical laboratory, where samples were stored at -20 C.

TABLE B.1. Study Use Pattern on Citrus Fruit [1].

Location (City, State) Year	Application						Tank Mix Adjuvants
	Timing	Formulation [2]	No. of Appl.	Actual Rate [3] (ppm)	Method [4]	App. volume (ml/min)	
Ontario, CA 2001	post-harvest	~3.2 lb/gal EC	1	681	dilute spray	490	None
				605	dilute spray	490	None
				622	dilute spray	490	None
				538	dilute spray	490	None
				534	dilute spray	490	None
				641	dilute spray	490	None

1 Both lemons and oranges were used in the first four test runs and only oranges were used in the last two tests.
2 Concentration in EC formulation (EPA Reg. No. 64864-31) was calculated by the reviewer.
3 Actual concentration of 2,4-D IPE in the treatment solution.
4 Following application of the dilute spray, fruit were waxed.

B.2 Analytical Methodology

The GC/MSD method used in this study (Method HWI 6578-101B) is essentially the same GC/MSD Method previously reviewed by the Agency (DP Barcode D222627, D. Miller, 6/11/96) for the enforcement of 2,4-D IPE tolerances in citrus commodities. Sample analyses for the current studies were performed by Convance Laboratories Inc., Madison, WI. A brief description of the GC/MSD method follows.

Residues in homogenized citrus fruit samples are extracted and hydrolyzed in 0.7 M NaOH for 1 hour at 100 C. The resulting extract is acidified and residues are partitioned into ethyl ether and dried by filtering through sodium sulfate. Residues are concentrated to dryness and then

methylated using boron trifluoride in methanol (70 C for 30 min). The methylated residues are then diluted with water, partitioned into hexane, and analyzed by GC/MSD using the m/z 234 ion for quantitation. Residues are quantified by direct comparison of peak areas to those of external standards and are reported in 2,4-D acid equivalents. The reported LOQ for 2,4-D IPE in/on citrus fruit is 0.05 ppm, but the method was validated to only 0.2 ppm.

C. RESULTS AND DISCUSSION

The number of post-harvest trials on citrus fruits is adequate. A total of 6 trial runs were conducted using a commercially available 3.2 lb/gal EC formulation of 2,4-D IPE. Four of the tests included both lemons and oranges and two of the tests used only oranges.

Total frozen (ca. -20 C) storage intervals for citrus fruit samples were ≤12 days (Table C. 2.), which is supported by the available storage stability data.

Based on the concurrent method recoveries, the GC/MSD method (HWI 6578-101B) is adequate for collecting data on 2,4-D IPE residues in/on citrus fruit. Concurrent method recoveries from orange and lemon samples fortified with 2,4-D IPE at 0.2-1.0 ppm were all within the acceptable 70-120% range, and average method recoveries were 97 ± 11% for oranges and 93 ± 12% for lemons (Table C.1.). Residues were also <0.05 ppm (<LOQ) in/on all 4 control samples. The validated LOQ for 2,4-D IPE is 0.2 ppm in/on citrus fruits; no LOD was reported. Adequate sample calculations and chromatograms were provided.

In six trail runs conducted in CA during 2001, 2,4-D IPE (3.2 lb/gal EC) was applied as a single, post-harvest, dilute aqueous spray to oranges (6 tests) and lemons (4 tests) at a concentration of 534-681 ppm (Table C.3.), followed by treatment with a waxing solution. The fruit were treated using standard commercial post-harvest application procedures. The study authors noted that citrus fruits can be treated with 2,4-D IPE either as a dilute spray prior to waxing or as a water-wax emulsion containing 2,4-D IPE. Although detailed data were not provided, preliminary data from trials conducted prior to writing the protocol indicated that 2,4-D residues were slightly higher following aqueous spray application (mean = 0.26 ppm) than following an application in wax (mean = 0.16 ppm). Therefore, extensive tests were conducted only on the aqueous spray.

Immediately following a post-harvest application of 2,4-D IPE as a dilute spray application, residues of 2,4-D were 0.158-0.242 ppm in/on 12 orange samples and 0.326-0.604 ppm in/on 8 lemon samples. Average 2,4-D residues were 0.200 ppm in/on oranges and 0.394 ppm in/on lemons.

TABLE C.1. Summary of Concurrent Recoveries of 2,4-D IPE from Citrus Fruits.

Matrix	Analyte	Spike level (mg/kg)	Sample size (n)	Recoveries (%)	Mean ± SD
Orange	2,4-D	0.20-1.0	4	84-109	97 ± 11
Lemon	2,4-D	0.20-1.0	4	81-110	93 ± 12

TABLE C.2. Summary of Freezer Storage Conditions

Matrix	Storage Temp. (°C)	Actual Storage Duration (days)	Limit of Demonstrated Storage Stability
Strawberry Fruit	-20 ± 10	6-12	7 months [1]

1 Storage stability data for citrus commodities was previously reviewed by the Agency (D221853, D. Miller, 7/8/96).

TABLE C.3. Residue Data from Citrus Post-harvest Trials with 2,4-D IPE.

Location [1] (City, State)Year	EPA [1] Region	Variety	Formulation	Actual Rate, ppm	Trial #	Residues (ppm)[2]
Ontario, CA, 2001	NA	Navel Orange	lb/gal EC	681	1	0.160, 0.242
				605	2	0.221, 0.219
				622	3	0.158, 0.199
				538	4	0.193, 0.211
				534	5	0.199, 0.212
				641	6	0.193, 0.188
Ontario, CA, 2001	NA	Lisbon Lemon	lb/gal EC	681	1	0.326, 0.604
				605	2	0.326, 0.406
				622	3	0.357, 0.368
				538	4	0.441, 0.327

1 As tests are for the post-harvest treatment of citrus, geographic representation is not relevant.
2 Each residue value is the average of two assays. Residues are expressed in 2,4-D acid equivalents.

TABLE C.4. Summary of Residue Data on Citrus Fruits from Post-harvest Trials with 2,4-D IPE.

Crop	Total Rate [1] (ppm)	PHI [2] (days)	Analyte	Sample size	Residue Levels (ppm) [3]				
					Min.	Max.	HAFT [4]	Mean	Std. Dev.
Oranges	604	na	2,4-D	12	0.158	0.242	0.220	0.200	0.024
Lemons	612	na	2,4-D	8	0.326	0.604	0.465	0.394	0.094
Both		na	2,4-D	20	0.158	0.604	0.465	0.278	0.115

1 Average actual concentration of 2,4-D IPE in application solutions.
2 Fruit were treated post-harvest and immediately sampled.
3 Residues are expressed in 2,4-D acid equivalents.
4 HAFT = Highest Average Field Trial.

D. CONCLUSION

The post-harvest citrus trial data are adequate and reflect the use of 2,4-D IPE (EC) as a dilute aqueous spray at a maximum rate of 500 ppm, which is 1x the currently labeled rate for lemons in the U.S. and for citrus fruits in other countries.

E. REFERENCES

DP Barcode: D221853
Subject: 2,4-D. (030001) Crop Field Trials and Processing Studies for Citrus (Orange, Grapefruit, and Lemon).
From: D. Miller
To: P. Deschamp
Date: 7/8/96
MRID(s): 43870301 through 43870303

DP Barcode: D222627
Subject: 2,4-D. (030066) Enforcement Analytical Method for IPE in Citrus Commodities.
From: D. Miller
To: P. Deschamp
Date: 6/11/96
MRID(s): 43893701

2,4-D Dimethylamine/PC Code: 30019/Riverdale Chemical Company
DACO 7.3/OPPTS 860.1380/OECD IIA 6.1.1 and IIIA 8.1.1
Storage Stability - Grape Matrices

Primary Evaluator	Dynamac Corporation 1910 Sedwick Rd. Durham, NC 27713 Contract No. 68-W-99-053	Date: 6/24/03
Reviewer	William J. Hazel, Ph.D., Chemist RRB1, HED (7509C)	Date: 3/1/04
Through	Whang Phang, Ph.D., Senior Scientist RRB1, HED (7509C)	

STUDY REPORTS:

45245601 Mester, T.; Fischer, E. (2000) Magnitude of the Residue of 2,4-D on Grape Raw Agricultural Products and Processed Commodities: Final Study Report: Lab Project Number: 97677:44086: 97677-A. Unpublished study prepared by ABC Laboratories California. 181 p.

EXECUTIVE SUMMARY:

In a storage stability study conducted concurrent with a grape field trial and processing study, control samples of whole grapes, grape juice, and raisins were fortified with 2,4-D at 0.5 ppm and stored frozen (-4 C) for up to 106 days (juice and raisins) or 273 days (grapes). Samples were analyzed using an adequate GC/ECD (EN-CAS Method No. ENC-2/93, with modifications) which has a limit of quantitation of 0.05 ppm for residues of 2,4-D in/on grape matrices.

The average corrected recovery of 2,4-D from stored samples (corrected for concurrent recoveries) was 81% for grapes stored 273 days, 103% for juice stored 106 days, and 109% for raisins stored 106 days. These data indicate that 2,4-D is stable at -20 in grapes for at least 9 months and in grape juice and raisins for at least 3.5 months.

STUDY/WAIVER ACCEPTABILITY/DEFICIENCIES/CLARIFICATIONS:

Under the conditions and parameters used in the study, the storage stability data are classified as scientifically acceptable. The storage stability data are adequate and support the concurrent grape field trial data reviewed in 45245601.der2.wpd. The acceptability of this study for regulatory purposes is also addressed in the Residue Chemistry Chapter of the 2,4-D RED [W. Hazel, 3/1/04, D287660].

COMPLIANCE:

Signed and dated GLP, quality assurance, and data confidentiality statements were provided. No deviations from regulatory requirements were noted that would impact the study results or their interpretation.

A. BACKGROUND INFORMATION

2,4-Dichlorophenoxyacetic acid (2,4-D) is a List A pesticide active ingredient classified as an herbicide, a plant growth regulator, and a fungicide. It is, however, mainly used as a selective postemergence herbicide for the control of certain weed species on a variety of food/feed sites including field, fruit, and vegetable crops. In addition to the parent acid, there are 8 salts and esters of 2,4-D, each with an assigned PC Code number, which are presently registered as active ingredients in herbicide end-use products (EPs).

The registrant submitted storage stability data in conjunction with grape field trial residue data supporting the use of 2,4-D dimethylamine (DMA) in grape vineyards.

TABLE A.1. Nomenclature of Test Compound

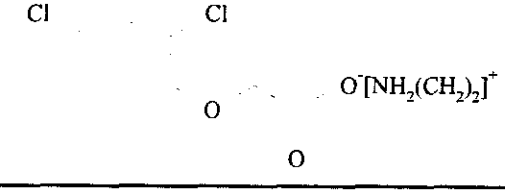
Compound	
Common name	2,4-D DMA
Company experimental names	None
IUPAC name	dimethylamine (2,4-dichlorophenoxy) acetate
CAS name	(2,4-dichlorophenoxy) acetic acid, dimethylamine salt
CAS #	2008-39-1
End-use products/EP	80.5% SC/S; EPA Reg. No. 288-260

TABLE A.2. Physicochemical Properties of 2,4-D DMA		
Parameter	Value	Reference (MRID)
Melting point/range	118-120 C	42829901
pH	6.8-9	not available
Density	1.23 g/cm^3	not available
Water solubility (25°C)	pH 5 321 g/L pH 7 729 g/L pH 9 664 g/L	not available
Solvent solubility (20°C)	acetonitrile 10.2 g/L methanol >500 g/L hexane 35.9 g/L 1-octanol 53.7 g/L toluene 1.65 g/L	not available
Vapor pressure at 25°C	<1.3 x 10^{-5} Pa	not available
Dissociation constant (pK_a)	3	not available
Octanol/water partition coefficient Log(K_{OW})	-0.83 at pH 7	not available
UV/visible absorption spectrum (λmax, nm)	not available	not available

B. EXPERIMENTAL DESIGN

B.1. Sample Preparation

Control samples of grapes, grape juice, and raisins fortified with 2,4-D at 0.5 ppm were placed in frozen storage at -20 C. Two subsamples were then extracted and analyzed within 4 days to establish "Day zero" recoveries. Stored samples of grapes were then analyzed after 106 and 273 days of frozen storage and juice and raisins were analyzed after 106 days of storage. At each storage interval, two stored samples and two freshly fortified samples were analyzed along with a control sample.

B.2. Analytical Methodology

Samples of grape matrices were analyzed for 2,4-D residues using the GC/ECD method EN-CAS Method No. ENC-2/93, with minor modifications. This method was previously validated and found to be adequate for data collection in/on various plant commodities (D. Miller, 1/24/96, CBRS No. 14004, DP Barcode D205346). A brief description of the method follows.

Residues are extracted into 0.5 M KOH in ethanol:H_2O (EtOH, 1:1, v/v) and filtered. The resulting extract is refluxed for 1 hour in 0.4 M HCl. Hydrolyzed residues are then cleaned-up using a C_{18} solid phase extraction column by rinsing with water and hexane, and then eluting residues with hexane:ethyl acetate (EtOAc, 1:1, v/v). Residues are concentrated to dryness and then derivatized to the methyl ester with diazomethane. The derivatized residues are then dissolved in 25% toluene in hexane and cleaned-up using an Alumina column eluted with 25% toluene in hexane. Methylated residues are determined by GC/ECD.

The analytical laboratory (ABC Laboratories, Madera, CA) validated the above GC/ECD method using control samples of grapes, grape juice, and raisins fortified with 2,4-D at 0.05, 0.50, and 1.0 ppm. The LOQ for 2,4-D is 0.05 ppm in/on each grape matrix. The LOD was not reported.

C. RESULTS AND DISCUSSION

The GC/ECD method is adequate for collecting data on residues of 2,4-D in grape matrices. Method validation recoveries averaged 81, 79, and 92% for whole grapes, juice, and raisins, respectively (Table C.1). In addition, recoveries from freshly fortified samples analyzed with the stored samples averaged 89% for grapes and 87% for juice and raisins. Apparent residues of 2,4-D were <0.05 ppm in all control samples.

Control samples of grapes, juice and raisins were fortified with 2,4-D at 0.5 ppm and stored at -20 C. The average corrected recovery of 2,4-D from stored samples (corrected for concurrent recoveries) was 81% for grapes stored 273 days, 103% for juice stored 106 days, and 109% for raisins stored 106 days (Table C.2).

TABLE C.1. Summary of Method Validation Recoveries of 2,4-D from Grape Matrices.

Matrix	Analyte	Spike level (ppm)	Sample size (n)	Recoveries (%) [1]	Mean ± std dev
Fruit	2,4-D	0.05-1.0	6	67-87 (1)	81 ± 7
Juice		0.05-1.0	6	76-85	79 ± 4
Raisins		0.05-1.0	5	85-102	92 ± 7

[1] The number of recoveries outside the acceptable 70-120% range is in parentheses.

TABLE C.2. Stability of 2,4-D in Grape Matrices Following Storage at -20°C.

Commodity	Spike level (mg/kg)	Storage interval (Days)	Recovery (%)		
			Stored Samples	Freshly Fortified	Corrected Average[1]
Whole fruit	0.5	0	81, 83 (82)	na	100
		106	81, 86 (84)	79, 83 (81)	104
		273	78, 79 (79)	94, 99 (97)	81
Juice	0.5	0	82, 84 (83)	na	100
		106	89, 91 (90)	85, 89 (87)	103
Raisins	0.5	0	84, 85 (85)	na	100
		106	97, 93 (95)	83, 90 (87)	109

[1] Average stored recovery was corrected for average procedural (freshly fortified) recovery.

D. CONCLUSION

The storage stability data are adequate and indicate that 2,4-D is stable in frozen (-20 C) grapes for up to 273 days (9 months) and in frozen grape juice and raisins for up to 106 days (3.5 months).

E. REFERENCES

CB No.: 14004
DP Barcode: D205346
Subject: 2,4-D. Enforcement Analytical Method for Plants. GDLN 171-4(c).
From: D. Miller
To: J. Coombs
Dated: 1/26/96
MRID(s): 43289301

2,4-D Dimethylamine/PC Code 30019/Riverdale Chemical Company
DACO 7.4.1/OPPTS 860.1500/OECD IIA 6.3.1, 6.3.2, 6.3.3 and IIIA 8.3.1, 8.3.2, 8.3.3
Crop Field Trial - Grape

Primary Evaluator	Dynamac Corporation 1910 Sedwick Rd. Durham, NC 27713 Contract No. 68-W-99-053	Date: 6/24/03
Reviewer	William J. Hazel, Ph.D., Chemist RRB1, HED (7509C)	Date: 3/1/04
Through	Whang Phang, Ph.D., Senior Scientist RRB1, HED (7509C)	

STUDY REPORTS:

45245601 Mester, T.; Fischer, E. (2000) Magnitude of the Residue of 2,4-D on Grape Raw Agricultural Products and Processed Commodities: Final Study Report: Lab Project Number: 97677:44086: 97677-A. Unpublished study prepared by ABC Laboratories California. 181 p.

EXECUTIVE SUMMARY:

In a total of 7 field trials conducted in CA (3 tests), NY (2 tests), and WA (2 tests) during 1997 and 1998, 2,4-D dimethylamine (80.5% ae SC/S) was applied as a single directed application to the soil in grape vineyards at 1.33-1.40 lb ae/A. The application was made in early spring around the time of bloom, prior to vines reaching the ground. Duplicate treated samples of mature grapes were collected from each test at 92-100 days after treatment.

Treated grape samples were stored frozen for a maximum of 193 days prior to analysis, an interval that is supported by the available stability data.

Residues of 2,4-D in/on grapes were determined using GC/ECD method EN-CAS Method No. ENC-2/93, with minor modifications. The method was validated and found to be adequate for data collection. For this method, residues are extracted from grapes with 0.5 M KOH in ethanol:H_2O (1:1, v/v), filtered, and refluxed for 1 hour in 0.4 M HCl. Hydrolyzed residues are then cleaned up using a C_{18} solid phase extraction column, concentrated to dryness and then derivatized to the methyl ester with diazomethane. The derivatized residues are then dissolved in 25% toluene in hexane, cleaned-up using an Alumina column eluted with 25% toluene in hexane, and analyzed by GC/ECD. The LOQ for 2,4-D is 0.05 ppm in/on grapes. The LOD was not reported.

Following a single broadcast application of 2,4-D DMA (SC/S) at 1.33-1.40 lb ae/A to the ground, residues of 2,4-D were <0.050 ppm in/on 14 samples of grapes harvested 92-100 days posttreatment.

STUDY/WAIVER ACCEPTABILITY/DEFICIENCIES/CLARIFICATIONS:

Although geographic representation of the field trial data was incomplete, the field trial residue data are classified as scientifically acceptable. Only seven grape field trials were conducted instead of the nine trials required under current guidance for grape tests having non-quantifiable residues. In addition, two tests were conducted in Region 11, instead of a single test in Regions 11 and 12.

The acceptability of this study for regulatory purposes is addressed in the Residue Chemistry Chapter of the 2,4-D RED [W. Hazel, 3/1/04, D287660].

COMPLIANCE:

Signed and dated GLP, quality assurance, and data confidentiality statements were provided. No deviations from regulatory requirements were noted that would impact the study results or their interpretation.

A. BACKGROUND INFORMATION

2,4-Dichlorophenoxyacetic acid (2,4-D) is a List A pesticide active ingredient classified as an herbicide, a plant growth regulator, and a fungicide. It is, however, mainly used as a selective postemergence herbicide for the control of certain weed species on a variety of food/feed sites including field, fruit, and vegetable crops. In addition to the parent acid, there are 8 salts and esters of 2,4-D, each with an assigned PC Code number, which are presently registered as active ingredients in herbicide end-use products (EPs).

To support the use of 2,4-D dimethylamine (DMA) in grape vineyards, the registrant has submitted data reflecting the use of 2,4-D DMA (80.5% ae SC/S) as a single directed application to weeds in grape vineyards at ~1.36 lb ae/A, prior to vines reaching the ground in spring.

TABLE A.1. Nomenclature of Test Compound

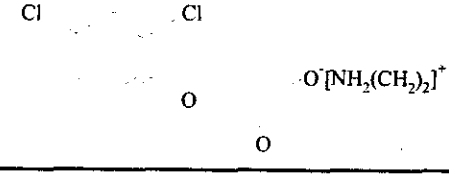
Compound	
Common name	2,4-D DMA
Company experimental names	None
IUPAC name	dimethylamine (2,4-dichlorophenoxy) acetate
CAS name	(2,4-dichlorophenoxy) acetic acid, dimethylamine salt
CAS #	2008-39-1
End-use products/EP	80.5% SC/S; EPA Reg. No. 288-260

TABLE A.2. Physicochemical Properties of 2,4-D DMA		
Parameter	Value	Reference (MRID)
Melting point/range	118-120 C	42829901
pH	6.8-9	not available
Density	1.23 g/cm^3	not available
Water solubility (25°C)	pH 5 321 g/L pH 7 729 g/L pH 9 664 g/L	not available
Solvent solubility (20°C)	acetonitrile 10.2 g/L methanol >500 g/L hexane 35.9 g/L 1-octanol 53.7 g/L toluene 1.65 g/L	not available
Vapor pressure at 25°C	<1.3 x 10^{-5} Pa	not available
Dissociation constant (pK_a)	3	not available
Octanol/water partition coefficient Log(K_{ow})	-0.83 at pH 7	not available
UV/visible absorption spectrum (λmax, nm)	not available	not available

B. EXPERIMENTAL DESIGN

B.1. Study Site Information

Information on cultural practices (cultivation and maintenance chemicals), soil characterization (Table B.1.1), and climatic data (temperature and rainfall) were provided for each trial location. The grapes were grown using standard cultural practices and no unusual weather conditions were noted during any of the trials. Temperatures at the test sites during 1997 and 1998 were normal compared to historical averages. Precipitation was also normal during 1997 and at one site during 1998. Two of the test sites in 1998 had above normal precipitation. Supplemental irrigation was used as needed.

TABLE B.1.1 Soil Characterization.				
Study Location (City, State), Year	Soil characteristics			
	Type	%OM	pH	CEC (meq/ 100 g)
Dundee, NY, 1997	Loam	3.1	5.7	11.7
Phelps, NY, 1997	Sandy Loam	1.1	4.3	7.0
Orland, CA, 1998	Loam	1.8	7.4	NR
Arbuckle, CA, 1998	Loam	1.2	7.0	16.6
Courtland, CA, 1998	Sandy Loam	1.2	6.6	13.6
Royal City, WA, 1997	Sandy Loam	1.0	8.1	15.4
George, WA, 1997	Sandy Loam	1.1	6.6	12.5

TABLE B.1.2. Study Use Pattern on Grapes.

Location (City, State) Year	Application						
	Formulation [1]	Timing [2]	Rate (lb a.e./A)	No. of Appl.	Method [3]	Volume (gal/A)	Tank Mix Adjuvants
Dundee, NY, 1997	80.5% SC/S	post-bloom	1.39	1	directed	20.5	None
Phelps, NY, 1997	80.5% SC/S	post-bloom	1.33	1	directed	19.6	None
Orland, CA, 1998	80.5% SC/S	pre-bloom	1.39	1	directed	26.7	None
Arbuckle, CA, 1998	80.5% SC/S	pre-bloom	1.40	1	directed	26.8	None
Courtland, CA, 1998	80.5% SC/S	40% bloom	1.36	1	directed	24.4	None
Royal City, WA, 1997	80.5% SC/S	3 mm fruit size	1.35	1	directed	19.9	None
George, WA, 1997	80.5% SC/S	3 mm fruit size	1.35	1	directed	19.9	None

[1] The 2,4-D DMA formulation is a 96.9% ai SC/S, which is equivalent to 80.5% ae.
[2] The applications were made in the spring prior to extensive vine growth.
[3] All applications were made using ground equipment and were made as a directed ground application to weeds growing under vines and between the rows.

TABLE B.1.3. Trial Numbers and Geographical Locations [1]

NAFTA Growing Region [1]	Total Grape Field Trials		
	Submitted	Requested	
		Canada	US [2]
1	2	NA	2
2	--	NA	--
3	--	NA	--
4	--	NA	--
5	--	NA	--
6	--	NA	--
7	--	NA	--
8	--	NA	--
9	--	NA	--
10	3	NA	8 (5)
11	2	NA	1
12	-	NA	1
Total	7	NA	12 (9)

[1] Regions 13-21 and 1A, 5A, 5B, and 7A were not included as the use is for the US only.
[2] Number of trials in parentheses is for 25% reduction in number of trials due to nonquantifiable residues.
NA = not applicable.

B.2. Analytical Methodology

The GC/ECD method EN-CAS Method No. ENC-2/93, with minor modifications, was used for determining residues of 2,4-D in/on grapes. This method was previously validated and found to be adequate for data collection in/on various plant commodities (D. Miller, 1/24/96, CBRS No. 14004, DP Barcode D205346). A brief description of the method follows.

Residues are extracted into 0.5 M KOH in ethanol:H_2O (1:1, v/v) and filtered. The resulting extract is refluxed for 1 hour in 0.4 M HCl. Hydrolyzed residues are then cleaned-up using a C_{18} solid phase extraction column by rinsing with water and hexane, and then eluting residues with hexane:ethyl acetate (1:1, v/v). Residues are concentrated to dryness and then derivatized to the methyl ester with diazomethane. The derivatized residues are then dissolved in 25% toluene in hexane and cleaned-up using an Alumina column eluted with 25% toluene in hexane. Methylated residues are determined by GC/ECD.

The analytical laboratory validated the above GC/ECD method using control samples of grapes fortified with 2,4-D at 0.05, 0.50, and 1.0 ppm. The LOQ for 2,4-D is 0.05 ppm in/on grapes. The LOD was not reported.

C. RESULTS AND DISCUSSION

In accordance with guidance for grape field trials having non-quantifiable residues, two tests were conducted in Region 1. However, only 3 tests were conducted in Region 10, instead of the required 5 tests, and 2 tests were conducted in Region 11, instead of one test each in Regions 11 and 12. Although the distribution of field trials was not fully representative and only 7 of the required 9 field trials were conducted, the number and geographic representation of the residue data on grapes are adequate given the use pattern.

Common cultural practices were used to maintain the vineyards, and the weather conditions and the maintenance chemicals used in the study did not have a notable impact on the residue data.

A single control and duplicate treated samples of mature grapes were collected from each test 92-100 days after application and immediately frozen. The supported PHI is 100 days for grapes. Samples were stored (<-10 C) at the field sites for 4-23 days prior to shipment by freezer truck to the analytical laboratory (ABC Laboratories California, Madera, CA), where samples were stored at <-20 C until analysis. The total frozen storage intervals were 93-193 days for treated grape samples (Table C.2). These storage intervals are supported by the available stability data (45245601.der1.wpd; W. Hazel, D276792, 3/1/04) which indicate that 2,4-D is stable in frozen grapes for at least 274 days.

The GC/ECD method (EN-CAS Method No. ENC-2/93) for determining residues of 2,4-D was validated by the analytical laboratory using control samples of grapes fortified at 0.05-1.0 ppm. Method validation recoveries averaged 81 ± 7% for grapes (Table C.1.1) and concurrent method recoveries averaged 90 ± 15% from 10 control samples fortified with 2,4-D at 0.05 or 0.5 ppm (Table C.1.2). Apparent residues of 2,4-D were <0.05 ppm in/on all control grape samples. The validated LOQ for 2,4-D is 0.05 ppm in/on grapes. The LOD was not reported. Adequate sample calculations and chromatograms were provided.

In a total of 7 field trials conducted in CA (3 tests), NY (2 tests), and WA (2 tests) during 1997

and 1998, 2,4-D DMA (80.5% ae SC/S) was applied as a single directed application to the soil at 1.33-1.40 lb ae/A. The application was made in early spring close to bloom, prior to vines reaching the ground. Residues of 2,4-D were <0.050 ppm in/on all 14 samples of grapes harvested ~100 days post-treatment (Table C.3).

TABLE C.1.1 Summary of Method Validation Recoveries of 2,4-D from Grape Matrices.

Matrix	Analyte	Spike level (ppm)	Sample size (n)	Recoveries (%) [1]	Mean ± std dev
Fruit	2,4-D	0.05-1.0	6	67-87 (1)	81 ± 7

[1] The number of recoveries outside the acceptable 70-120% range is in parentheses.

TABLE C.1.2 Summary of Concurrent Recoveries of 2,4-D from Grapes.

Matrix	Analyte	Spike level (ppm)	Sample size (n)	Recoveries (%)	Mean ± std dev
Grape	2,4-D	0.05	6	79-85	83 ± 3
		0.5	4	71-123 (1)	94 ± 19

[1] The number of recoveries outside the acceptable 70-120% range is in parentheses.

TABLE C.2. Summary of Storage Conditions

Matrix (RAC)	Storage Temp. (C)	Actual Storage Duration from Harvest to Analysis (days)	Limit of Demonstrated Storage Stability (days) [1]
Grape Fruit	-20	93-193	274

[1] A storage stability study was conducted by the analytical laboratory concurrently with the field trials. Storage stability data are reported in 45245601.der1.wpd (W. Hazel, D276792, 3/1/04).

TABLE C.3. Residue Data on Grapes from Field Trials with 2,4-D DMA.

Location (City, State), Year	EPA Region	Variety	Total Rate (lb ae/A)	PHI (days)	2,4-D Residues (ppm) [1]
Dundee, NY, 1997	1	DeChaunac	1.39	100	<0.050, <0.050
Phelps, NY, 1997	1	Catawba	1.33	100	<0.050, <0.050
Orland, CA, 1998	10	Barbera	1.39	100	<0.050, <0.050
Arbuckle, CA, 1998	10	Zinfindel	1.40	100	<0.050, <0.050
Courtland, CA, 1998	10	Chardonnay	1.36	100	<0.050, <0.050
Royal City, WA, 1997	11	Cabernet Franc	1.35	92	<0.050, <0.050
George, WA, 1997	11	Chardonnay	1.35	92	<0.050, <0.050

[1] The LOQ is 0.05 ppm.

D. CONCLUSION

Although the distribution of field trials was not fully representative and only 7 of the required 9 field trials were conducted, the grape field trial data are adequate and reflect the use of 2,4-D DMA (SC/S) at a maximum seasonal application rate of 1.36 lb ae/A, which is 1x the use rate for grapes.

E. REFERENCES

CBRS No.: 14004
DP Barcode: D205346
Subject: Enforcement Analytical Method for Plants.
From: D. Miller
To: J. Coombs
Date: 1/24/96
MRID(s): 43289301

Template Version March 2003

2,4-D Dimethylamine/PC Code: 30019/Riverdale Chemical Company
DACO 7.4.1/OPPTS 860.1500/OECD IIA 6.3.1, 6.3.2, 6.3.3 and IIIA 8.3.1, 8.3.2, 8.3.3
Processed Food and Feed - Grape

Primary Evaluator	Dynamac Corporation 1910 Sedwick Rd. Durham, NC 27713 Contract No. 68-W-99-053	Date: 6/25/03
Reviewer	William J. Hazel, Ph.D., Chemist RRB1, HED (7509C)	Date: 3/1/04
Through	Whang Phang, Ph.D., Senior Scientist RRB1, HED (7509C)	

STUDY REPORT:

45245601 Mester, T.; Fischer, E. (2000) Magnitude of the Residue of 2,4-D on Grape Raw Agricultural Products and Processed Commodities: Final Study Report: Lab Project Number: 97677:44086: 97677-A. Unpublished study prepared by ABC Laboratories California. 181 p.

EXECUTIVE SUMMARY:

In one test conducted in NY during 1997, 2,4-D dimethylamine (80.5% ae SC/S) was applied as a broadcast ground application in a vineyard at 1.39 lb ae/A. The application was made immediately prior to bloom, before substantial vine growth. Two separate bulk samples of treated grapes were collected at 100 days post-treatment. These samples were separately processed into grape juice and raisins; however, no details of the processing procedures were provided. Samples of grapes, juice, and raisins were stored frozen for 93-99 days prior to analysis, an interval supported by available stability data.

Grape matrices were analyzed for residues of 2,4-D using GC/ECD method EN-CAS Method No. ENC-2/93, with minor modifications. The method was validated and found to be adequate for data collection. For this method, residues are extracted from grape matrices with 0.5 M KOH in ethanol:H_2O (1:1, v/v), filtered, and refluxed for 1 hour in 0.4 M HCl. Hydrolyzed residues are then cleaned-up using a C_{18} solid phase extraction column, concentrated to dryness and then derivatized to the methyl ester with diazomethane. The derivatized residues are then dissolved in 25% toluene in hexane, cleaned-up using an Alumina column eluted with 25% toluene in hexane, and analyzed by GC/ECD. The LOQ for 2,4-D is 0.05 ppm in/on grapes, grape juice, and raisins. The LOD was not reported.

Following a single early-season broadcast application of 2,4-D DMA (SC/S) to the vineyard floor at 1.39 lb ai/A, residues of 2,4-D were <0.05 ppm in/on all 4 samples of grapes and <0.05 ppm in 4 samples of juice and 4 samples of raisins derived from the treated grapes. As residues were <LOQ, processing factors could not be determined.

STUDY/WAIVER ACCEPTABILITY/DEFICIENCIES/CLARIFICATIONS:

Under the conditions and parameters used in the study, the grape processing study is classified as scientifically unacceptable. In addition, the study can not be upgraded. The following deficiencies were noted in this grape processing study.

- Information on where and how the grapes were processed was not provided.
- Although residues were <LOQ in all grape samples used for processing and in the resulting grape juice and raisin samples, the field trail was conducted at only 1x the maximum labeled use rate.

A processing study should be conducted using an exaggerated application rate as residues in grapes are expected to be <LOQ following treatment at the maximum labeled rate. As grapes have the theoretical concentration factor of 4.7x for raisins, the grape processing study should use an application at 5x the maximum labeled rate.

The acceptability of this study for regulatory purposes is also addressed in the Residue Chemistry Chapter of the 2,4-D RED [W. Hazel, 3/1/04, D287660].

COMPLIANCE:

Signed and dated GLP, quality assurance, and data confidentiality statements were provided. No deviations from regulatory requirements were noted that would impact the study results or their interpretation.

A. BACKGROUND INFORMATION

2,4-Dichlorophenoxyacetic acid (2,4-D) is a List A pesticide active ingredient classified as an herbicide, a plant growth regulator, and a fungicide. It is, however, mainly used as a selective postemergence herbicide for the control of certain weed species on a variety of food/feed sites including field, fruit, and vegetable crops. In addition to the parent acid, there are 8 salts and esters of 2,4-D, each with an assigned PC Code number, which are presently registered as active ingredients in herbicide end-use products (EPs).

To support the use of 2,4-D dimethylamine (DMA) in grape vineyards, the registrant has submitted a data reflecting the potential for 2,4-D residues occurring in processed fractions derived from grapes grown in vineyards treated with of 2,4-D DMA (80.5% ae SC/S) as a single directed ground application at 1.39 lb ae/A, prior to vines reaching the ground in spring.

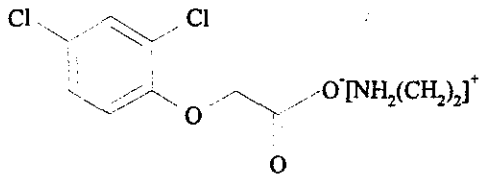
TABLE A.1. Nomenclature of Test Compound	
Compound	
Common name	2,4-D DMA
Company experimental names	None
IUPAC name	dimethylamine (2,4-dichlorophenoxy) acetate
CAS name	(2,4-dichlorophenoxy) acetic acid, dimethylamine salt
CAS #	2008-39-1
End-use products/EP	80.5% SC/S; EPA Reg. No. 288-260

TABLE A.2. Physicochemical Properties of 2,4-D DMA		
Parameter	Value	Reference (MRID)
Melting point/range	118-120 C	42829901
pH	6.8-9	not available
Density	1.23 g/cm^3	not available
Water solubility (25°C)	pH 5 321 g/L pH 7 729 g/L pH 9 664 g/L	not available
Solvent solubility (20°C)	acetonitrile 10.2 g/L methanol >500 g/L hexane 35.9 g/L 1-octanol 53.7 g/L toluene 1.65 g/L	not available
Vapor pressure at 25°C	<1.3 x 10^{-5} Pa	not available
Dissociation constant (pK$_a$)	3	not available
Octanol/water partition coefficient Log(K$_{ow}$)	-0.83 at pH 7	not available
UV/visible absorption spectrum (λmax, nm)	not available	not available

B. EXPERIMENTAL DESIGN

B.1. Application and Crop Information

TABLE B.1.2. Study Use Pattern on Grapes.

Location (City, State) Year	Application						
	Formulation [1]	Timing [2]	Rate (lb a.e./A)	No. of Appl.	Method [3]	Volume (gal/A)	Tank Mix Adjuvants
Dundee, NY, 1997	80.5% SC/S	pre-bloom	1.39	1	directed	20.5	None

[1] The 2,4-D DMA formulation is a 96.9% ai SC/S, which is equivalent to 80.5% ae.
[2] The application was made in the spring prior to extensive vine growth.
[3] All applications were made using ground equipment and were made as a directed ground application to weeds growing under and between the rows.

B.2. Processing Procedures

After harvest the bulk samples were held at 1-4 C for 6 days prior to processing. The two bulk samples of grapes were separately processed into juice and raisins; however, no details of the processing procedures were provided. After processing, samples of grapes, juice, and raisins were frozen and shipped by freezer truck to the analytical laboratory (ABC Laboratories, Madera, CA), where the samples were stored at -20 C.

B.3. Analytical Methodology

The GC/ECD method EN-CAS Method No. ENC-2/93, with minor modifications, was used for determining residues of 2,4-D in/on grapes and grape processed fractions. This method was previously validated and found to be adequate for data collection in/on various plant commodities (D. Miller, 1/24/96, CBRS No. 14004, DP Barcode D205346). A brief description of the method follows.

Residues are extracted into 0.5 M KOH in ethanol:H_2O (1:1, v/v) and filtered. The resulting extract is refluxed for 1 hour in 0.4 M HCl. Hydrolyzed residues are then cleaned-up using a C_{18} solid phase extraction column by rinsing with water and hexane, and then eluting residues with hexane:ethyl acetate (1:1, v/v). Residues are concentrated to dryness and then derivatized to the methyl ester with diazomethane. The derivatized residues are then dissolved in 25% toluene in hexane and cleaned-up using an Alumina column eluted with 25% toluene in hexane. Methylated residues are determined by GC/ECD.

The analytical laboratory validated the above GC/ECD method using control samples of grapes, juice, and raisins each fortified with 2,4-D at 0.05, 0.50, and 1.0 ppm. The LOQ for 2,4-D is 0.05 ppm in/on each commodity. The LOD was not reported.

C. RESULTS AND DISCUSSION

In one test conducted in NY during 1997, 2,4-D dimethylamine (80.5% ae SC/S) was applied as a single directed ground application to a vineyard at 1.39 lb ae/A. The application was made immediately prior to bloom, before substantial vine growth. Two separate bulk samples of treated grapes were collected at 100 days post-treatment.

After harvest the bulk samples were held at 1-4 C for 6 days prior to processing. The two bulk samples of grapes were separately processed into juice and raisins; however, no details of the processing procedures were provided. After processing, samples of grapes, juice, and raisins were frozen and shipped by freezer truck to the analytical laboratory (ABC Laboratories, Madera, CA), where the samples were stored at -20 C. The total frozen storage interval for the grapes and processed fractions was 93-99 days; an interval that is supported by the available stability data (45245601.der1.wpd; W. Hazel, D276792, 3/1/04).

Grape matrices were analyzed for residues of 2,4-D using GC/ECD method EN-CAS Method No. ENC-2/93, with minor modifications. The method was validated by the analytical laboratory using control samples of grapes, juice, and raisins fortified at 0.05-1.0 ppm. Method validation recoveries averaged 81 ± 7% for grapes, 79 ± 4% for juice, and 92 ± 7% for raisins (Table C.1.1). Concurrent method recoveries averaged 82, 85, and 89% for control samples of grapes, juice, and raisins, respectively, fortified with 2,4-D at 0.05 or 0.5 ppm (Table C.1.2). Apparent residues of 2,4-D were <0.05 ppm in/on all control samples. The validated LOQ for 2,4-D is 0.05 ppm in/on grape matrices. The LOD was not reported. Adequate sample calculations and chromatograms were provided.

Following a single early-season directed application of 2,4-D DMA (SC/S) to the vineyard floor at 1.39 lb ai/A, residues of 2,4-D were <0.05 ppm in/on all 4 samples of grapes and in 2 samples each of juice and raisins derived from the treated grapes. As residues were <LOQ, processing factors could not be determined.

TABLE C.1.1 Summary of Method Validation Recoveries of 2,4-D from Grape Matrices.

Matrix	Analyte	Spike level (ppm)	Sample size (n)	Recoveries (%) [1]	Mean ± std dev
Whole fruit	2,4-D	0.05-1.0	6	67-87 (1)	81 ± 7
Juice		0.05-1.0	6	76-85	79 ± 4
Raisins		0.05-1.0	5	85-102	92 ± 7

[1] The number of recoveries outside the acceptable 70-120% range is in parentheses.

TABLE C.1.2 Summary of Concurrent Recoveries of 2,4-D from Grapes and Grape Processed Fractions.					
Matrix	Analyte	Spike level (ppm)	Sample size (n)	Recoveries (%)	Mean ± std dev
Whole fruit	2,4-D	0.05, 0.5	4	80, 123, 79, 84	92 ± 21
Juice		0.05, 0.5	2	90, 79	85
Raisins		0.05, 0.5	2	94, 83	89

[1] The number of recoveries outside the acceptable 70-120% range is in parentheses.

TABLE C.2. Summary of Freezer Storage Conditions			
Apple Matrix	Storage Temp. (°C)	Actual Storage Duration (days)	Limit of Demonstrated Storage Stability (days) [1]
Whole fruit	-20	96-99	273
Juice		93	106
Raisins		96	106

[1] The submitted frozen storage stability data for grapes, juice, and raisins are reviewed in 45245601.der1.

TABLE C.3. Residue Data from Grape Processing Study with 2,4-D DMA.					
RAC	Processed Commodity	Total Rate [1] (lb ae/A)	PTI (days)	2,4-D Residues (ppm) [2]	Processing Factor
Grape	whole grapes	1.39	100	<0.05, <0.05, <0.05, <0.05	NA
	Juice			<0.05, <0.05	NA
	Raisins			<0.05, <0.05	NA

[1] The 1x rate for grapes is 1.36 lb ae/A.
[2] The LOQ is 0.05 ppm.
NA = not applicable

D. CONCLUSION

The grape processing study is not adequate as information pertaining to the processing procedures was not provided. In addition, although residues were <LOQ in all grape samples used for processing and in the resulting grape juice and raisin samples, the field trail was conducted at only the 1x the maximum labeled rate. Agency guidance requires use of exaggerated application rates for processing studies where residues in the RAC are expected to be <LOQ following treatment at the maximum labeled rate. In the case of grapes, which have a theoretical concentration factor of 4.7x for raisins, the grape processing study should use an application at 5x the maximum labeled rate.

E. REFERENCES

CB No.: 14004
DP Barcode: D205346
Subject: 2,4-D. Enforcement Analytical Method for Plants. GDLN 171-4(c).
From: D. Miller
To: J. Coombs
Dated: 1/26/96
MRID(s): 43289301

2,4-D
Case 0073; PC Code 030001
(DP Barcode D283959)

Registrant's Response to Product Chemistry Data Requirements

July 8, 2003

Contract No. 68-W-99-053

Submitted to:
U.S. Environmental Protection Agency
Arlington, VA

Submitted by:
Dynamac Corporation
20440 Century Blvd, Suite 100
Germantown, MD 20874

REVIEW OF PRODUCT CHEMISTRY, OPPTS 830 SERIES

Chemical Name (IUPAC, ANSI, etc.)	2,4-D; 2,4-dichlorophenoxy acetic acid
Chemical Number (CAS; PC Code)	CAS No. 94-75-7 PC Code 030001
Registration No.	
Type of Product (T, FI, MP, EP)	TGAI
DP Barcode	D283959

Product chemistry data (2002; MRID 45692501) were submitted by the Industry Task Force II on 2,4-D Research Data for the 2,4-D acid TGAI/PAI. The Task Force had determined that previously submitted water solubility data (MRID 41332002) did not represent the actual solubility of 2,4-D acid *per se* because the pH was adjusted with sodium hydroxide or sodium borate, producing salts of 2,4-D which had much higher solubility than the acid *per se*.

The new study was conducted by Wildlife International, Ltd. (Easton, MD) using the shake-flask method. Subsamples of the 2,4-D acid TGAI/PAI (99.5% purity) were dissolved in NANOpure® water, and 2,4-D was quantitated by HPLC/UV. The solubility of 2,4-D acid in water was found to be 569 mg/L at 20 C. These data supercede previously submitted water solubility data for 2,4-D acid, and will support all 2,4-D acid products registered to Task Force members (currently Dow AgroSciences LLC, Agro-Gor Corporation, BASF Aktiengesellschaft, and NuFarm USA, Inc.).

Because the data are representative of a single characteristic of the TGAI/PAI rather than an individual product, no data tables were prepared for this review.

2,4-D
PC Code 030001; Case 0073
(DP Barcode D276792)

Registrant's Response to Residue Chemistry Data Requirements

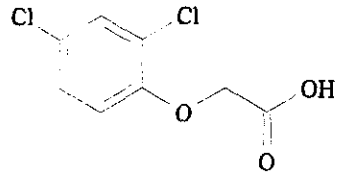
August 11, 2000

Contract No. 68-W-99-053

Submitted to:
U.S. Environmental Protection Agency
Arlington, VA

Submitted by:
Dynamac Corporation
1910 Sedwick Road
Building 100, Suite B
Durham, NC 27713

2,4-D



(PC Code 030001; Case No. 0073)

DP Barcode D276792

REGISTRANT'S RESPONSE TO RESIDUE CHEMISTRY DATA REQUIREMENTS

BACKGROUND

In response to outstanding data requirements for reregistration of 2,4-D, the Industry Task Force II on 2,4-D Research Data (TF II) submitted data pertaining to residues in meat and milk of dairy cattle (1996, MRID 44424801). A protocol for this study was previously approved by HED (DP Barcode D216135, 6/15/95, S. Knizner). These data are intended to support the established tolerances for 2,4-D residues in ruminant commodities. The Conclusions and Recommendations stated in this review pertain only to the magnitude of the residue in livestock.

The nature of the residue in plants is adequately understood based upon acceptable wheat, lemon, and potato metabolism studies. The nature of the residue in animals is understood based upon acceptable ruminant and poultry metabolism studies.

Tolerances for residues of 2,4-D (2,4-dichlorophenoxyacetic acid) in/on plant commodities and fish are expressed in terms of 2,4-D *per se* [40 CFR §180.142(a)(1-6, 9-12) and (b)]. Tolerances for residues in livestock commodities are currently established in terms of residues of 2,4-D and/or its metabolite 2,4-dichlorophenol [40 CFR §180.142(a)(8)]. The HED Metabolism Assessment Review Committee (MARC), on 9/3/03, concluded that the residue of concern in plants and animals is 2,4-D, both free and conjugated, determined as the acid (W. Hazel and L. Taylor, 12/3/03, TXR No. 0052264, D293119). It was also determined that 2,4-dichlorophenol should be removed from the residue definition of 2,4-D tolerances in livestock commodities. Adequate methods are available for data collection. A proposed GC/ECD enforcement method for animal commodity tolerances has been approved contingent upon receipt of additional information required by HED (DP Barcode D226556, 6/26/96, D. Miller).

Codex MRLs (CXL) of 0.05 ppm (at or about the limit of detection) are established for 2,4-D

residues in eggs, meat (from mammals other than marine mammals), milk products and milks. These MRLs are lower than the established U.S. tolerances. Issues regarding the compatibility of the U.S. tolerances and Codex MRLs have been addressed in the Residue Chemistry Chapter of the 2,4-D RED [W. Hazel, 3/1/04, D287660].

CONCLUSIONS AND RECOMMENDATIONS

1. An adequate GC/ECD method was used for data collection in the submitted cattle feeding study. This method is adequate for tolerance enforcement, contingent upon receipt of additional information requested by HED (DP Barcode D226556, 6/26/96, D. Miller). The registrants must provide the following:

   (i) submit a revised method which combines the two methods into a single method and, once an adequate revised method is received, the Agency will forward the method to EPA/BEAD/ACB/Ft. Meade for a tolerance method validation; (ii) the analytical method instructions should be modified to delete all references to the use of diazomethane as a derivatizing agent; and (iii) provide to the Agency complete raw data and sample calculations (including chromatograms showing peak areas, external standard linearity curves and associated data, standard calculations, etc.). Recently, it has been determined that the technology to generate diazomethane has advanced such that the compound may be generated *in situ* at dilute concentrations. This newer technique is not considered to be a dangerous procedure. However, some laboratories remain hesitant to use this explosive and carcinogenic derivatizing agent at all whereas others prefer the older technique. As a result, the use of diazomethane as a derivatizing agent in a regulatory method, while still discouraged, is now considered acceptable (minutes of 9/17/03 HED/ChemSAC meeting). Therefore, resolution of item (ii) above is now considered to be optional.

2. Adequate storage stability data were submitted with the feeding study. Untreated samples were fortified with 2,4-D at 1.0 ppm and analyzed after 0, 57-81, and 106-132 days of frozen storage. Adequate recoveries were obtained from liver, kidney, muscle, and milk at each storage interval. Study samples were stored for up to 4 months prior to analysis.

3a. The submitted cattle feeding study is adequate. Four groups of three cows each were dosed orally with 2,4-D at nominal rates of 1500, 3000, 6000, and 9000 ppm for 28-30 days, equivalent to 1.7, 3.4, 6.8, and 10.2x the maximum theoretical dietary burden for cattle, and were sacrificed within 20 hours of the final dose. Two additional groups of cows were dosed at the 10.2x level and sacrificed 3 or 7 days after the cessation of dosing. Milk, liver, kidneys, composite muscle (round and tenderloin), and composite fat (perirenal and omental) were collected.

3b. Residues in milk plateaued after 7-11 days of dosing. Maximum residues were 0.07 ppm at the low dose level (1.7x). At the 3.4, 6.8, and 10.2x dose levels, maximum milk residues

were 0.18, 0.58, and 0.87 ppm, respectively. After the 3- and 7-day recovery periods, residues in milk from the 10.2x dose group declined to <0.01-0.02 ppm.

3c. Maximum residues at the low dose (1.7x) were 0.20 ppm in liver, 6.48 ppm in kidney, 0.24 ppm in muscle, and 0.51 ppm in fat. Immediately following dosing (0-day PSI), maximum residues at the high dose (10x) were 3.80 ppm in liver, 24.4 ppm in kidney, 1.02 ppm in muscle, and 2.30 ppm in fat. After a 3-day recovery from dosing at 9000 ppm, residues had declined to 0.67 ppm in liver, 0.1 ppm in kidney, 0.06 ppm in muscle, and 0.12 ppm in fat. After 7 days recovery, residues were 0.51 ppm in liver and <0.05 ppm in kidney, muscle, and fat.

3d. The submitted cattle feeding study is adequate. Assuming a 0-day PGI and extrapolating the data from the low-dose group (1.7x) to a 1-x feeding level; the established 0.1 ppm tolerance for 2,4-D residues in milk can be lowered to 0.05 ppm, equivalent to the Codex MRL for milk (0.05 mg/kg). However, the established tolerances of 0.2 ppm for 2,4-D residues in fat, meat, and meat byproducts (excluding kidney) of cattle, goats, horses, and sheep are insufficient and should be increased to 0.3 ppm, and the established 2-ppm tolerance for residues in kidney should be increased to 4 ppm.

DETAILED CONSIDERATIONS

OPPTS GLN 860.1360: Residue Analytical Methods

Residues in cattle tissues and milk were analyzed using the proposed enforcement method for ruminant tissues. In this method, samples are refluxed in 2N HCl. The aqueous hydrolyzed extract is diluted with ACN and is subsequently cleaned up on a Florisil column to remove matrix interferences. The resultant extract is diluted with 1% NaOH and acidified following rotary evaporation, and residues are partitioned into a solution of 10% EtOAc in hexane. The organic phase is passed through a neutral alumina column and the analyte eluted with a solution of NaOH in MeOH. The extract is acidified and partitioned with methyl-*tert*-butyl ether (MTBE), the MTBE layer is concentrated, and the residues are methylated with BF_3/MeOH. Derivatized residues are analyzed by GC/ECD. Recoveries when residues were derivatized with BF_3/MeOH were 62.9-118% from muscle, 62.6-97.2% from fat, 60.8-102% from kidney, 63.5-117% from liver, and 89.9-119 % from milk. These data are tabulated in the HED review of the method (DP Barcode D226556, 6/26/96, D. Miller).

This method is adequate for data collection and tolerance enforcement, contingent upon receipt of the additional information required by HED (DP Barcode D226556, 6/26/96, D. Miller).

OPPTS GLN 860.1380: Storage Stability Data

Storage stability data were submitted with this feeding study. Untreated samples of cattle matrices were fortified with 2,4-D at 1.0 ppm and analyzed at the beginning of storage and after approximately 2 and 4 months. The results are presented in Table 1. 2,4-D residues are stable in edible cattle matrices stored frozen for the intervals observed in the feeding study.

Table 1. Storage stability of 2,4-D residues in cattle samples fortified at 1.0 ppm.

Matrix	Storage Interval (days)	Fresh Fortification Recovery (%)	Recovery from Stored sample (%)	Corrected Recovery in Stored Sample (%)
Liver	0	99.4	--	--
	77	97.9	112.4	114.8
	130	101.6	108.3	106.5
Kidney	0	71.3	--	--
	65	101.0	113.0	111.8
	121	75.1	93.1	123.9
Muscle	0	110.9	--	--
	67	106.2	112.7	106.1
	115	109.0	120.4	110.4
Fat	0	84.6	--	--
	57	93.7	104.5	111.5
	106	74.3	86.8	116.8
Milk	0	71.2	--	--
	81	99.5	80.7	81.1
	132	99.1	107.7	108.7

OPPTS GLN 860.1480: Magnitude of the Residue in Animals

The following tolerances for residues in animal commodities are currently established in terms of residues of 2,4-D and/or its metabolite 2,4-dichlorophenol [40 CFR §180.142 (a)(8)]: 0.2 ppm in the fat, meat, and meat byproducts (except kidney) of cattle, goats, hogs, horses, and sheep; 2 ppm in the kidney of cattle, goats, hogs, horses, and sheep; and 0.1 ppm in milk.

Maxium theoretical dietary burden (MTDB). The MTDB for beef and dairy cattle is 881 ppm, based on a diet consisting of pasture or range grass forage, aspirated grain fractions, and grain milled fractions. Calculations based on estimated reassessed tolerances are presented in Table 2. The label directions for 2,4-D use on grass specify a 3-day preslaughter interval (PSI) for cattle grazed on treated areas. The next highest possible dietary burden for cattle would result from

substituting wheat forage for grass forage; the resulting dietary burden would be 160 ppm and the lowest feeding level would be approximately 10x.

Table 2. Theoretical dietary burden concentrations of 2,4-D for beef and dairy cattle, based on reassessed tolerances.

Feed item	Percent dry matter	Reassesed Tolerances (ppm) [a]	Percent in diet (dairy/beef)	Dietary burden (ppm) [b]
Grass forage	25	360	60	864
Aspirated grain fractions	85	60	20	15
Grain milled fractions	88	10	20	2.3
Total			**100**	**881**
Additional feed items with tolerances for 2,4-D residues				
Apple pomace	40	0.05	20/40	0.03
Citrus pulp (dried)	91	3.0	20	0.66
Corn forage	40	6.0	50/40	7.5
Corn grain	88	0.05	40/80	0.02
Corn stover	83	70	15/25	13
Potatoes	20	0.2	40/75	0.4
Rice bran	90	0.5	15	0.08
Rice grain	88	0.5	40	0.4
Rice hulls	90	1.5	10	0.17
Rice straw	90	10	10	1.1
Sorghum forage	35	0.2	50/40	0.29
Sorghum grain	86	0.05	40	0.02
Sorghum stover	86	0.05	15/25	0.01
Sugarcane molasses	75	0.2	10	0.03
Wheat grain	89	2.0	40/50	0.90
Wheat forage	25	60	25/60	60 (144 beef)

[a] Tolerance levels are based on recommendations from the Residue Chemistry Chapter of the 2,4-D RED (W. Hazel, 3/1/04, D287660).

[b] Dietary burdens calculated based on percentages of dairy feed items, unless otherwise indicated.

Cattle Feeding Study. The TF II submitted data (1996, MRID 44024801) pertaining to 2,4-D residues in milk and tissues of cows dosed with 2,4-D for 28-30 days. Four groups of three cows each were dosed orally with 2,4-D at nominal rates of 1,500, 3,000, 6,000, and 9,000 ppm in the diet. Actual doses were 1,446, 2,890, 5,779, and 8,585 ppm. These dose levels are approximately equivalent to 1.7x, 3.4x, 6.8x, and 10x the MTDB of 881 ppm, most of which is based on pasture/range grass forage (25% dry matter) at a reassessed tolerance level of 360 ppm for a 0-day PHI/PGI, aspirated grain fractions, and grain milled fractions.

Milk was collected twice daily throughout the study and composite samples were prepared from each cow for days 0, 1, 3, 7, 11, 14, 18, 21, 24, and 28. The animals were sacrificed 12-18 hours after the final dose. Two additional groups of three cows dosed at the highest level (9000 ppm) were sacrificed 3 or 7 days after the cessation of dosing. Liver, kidneys, composite muscle (round and tenderloin), and composite fat (perirenal and omental) were collected, chilled, and shipped to the analytical facility, where they were chopped, homogenized and stored frozen. Samples were stored for up to 4 months prior to analysis. Residues were analyzed using the proposed enforcement method described above. Residue data are presented in Table 3.

Residues in milk plateaued after 7-11 days of dosing. Maximum residues were 0.07 ppm at the low dose level. At 3.4, 6.8, and 10x doses, maximum milk residues were 0.18, 0.58, and 0.87 ppm. Residues in milk from the 9000 ppm dose had decreased to <0.01-0.02 after 3 and 7 days recovery.

Maximum residues at the low dose (1.7x) were 0.20 ppm in liver, 6.48 ppm in kidney, 0.24 ppm in muscle, and 0.51 ppm in fat. Immediately following dosing (0-day PSI), maximum residues at the high dose (10x) were 3.80 ppm in liver, 24.4 ppm in kidney, 1.02 ppm in muscle, and 2.30 ppm in fat. After a 3-day recovery from dosing at 9000 ppm, residues had declined to 0.67 ppm in liver, 0.1 ppm in kidney, 0.06 ppm in muscle, and 0.12 ppm in fat. After 7 days recovery, residues were 0.51 ppm in liver and <0.05 ppm in kidney, muscle, and fat.

The submitted cattle feeding study is adequate. Assuming a 0-day PSI and using the data from the low-dose group (1.7x), the established 0.1 ppm tolerance for 2,4-D residues in milk can be lowered to 0.05 ppm, equivalent to the Codex MRL for milk (0.05 mg/kg). However, the established tolerances of 0.2 ppm for 2,4-D residues in fat, meat, and meat byproducts (excluding kidney) of cattle, goats, hogs, horses, and sheep are insufficient and should be increased to 0.3 ppm, and the established 2 ppm tolerance for residues in kidney should be increased to 4 ppm.

However, HED notes that residues of 2,4-D were excreted rapidly by livestock, as evidenced by the residue decline in tissues following withdrawal from the high-dose (9,000x). Based on average residues in tissues from the high-dose groups, 2,4-D residues declined by 85% in liver, >99% in kidneys, 94% in muscle, and 97% in fat following a 3-day withdrawal period. If a 3-day PSI is allowed for livestock grazing on treated grass forage (pasture and rangeland uses), then tolerances for 2,4-D in fat, meat, and meat byproducts could be lowered to 0.05 ppm, even

considering the relative high dietary burden. This would also result in the harmonization of the U.S. tolerances and with the Codex MRLs for livestock commodities.

Table 3. Residues of 2,4-D in milk and cow tissues during the 28-day study and after a 3- or 7-day recovery period.

Residues (ppm) [a]						
Martix/ Day	0-day Pre-slaughter Interval (PSI)				3-day PSI	7-day PSI
	1500 ppm (1.7x) [b]	3000 ppm (3.4)	6000 ppm (6.8x)	9000 ppm (10x)	9000 ppm (10x)	9000 ppm (10x)
Milk						
0	<0.01-0.01	<0.01	<0.01-0.01	<0.01, <0.01	<0.01	<0.01
1	0.02-0.03	0.09-0.18	0.16-0.31	0.30-0.31	N/A [c]	N/A
3	0.02-0.04	0.06-0.18	0.11-0.39	0.35-0.37	N/A	N/A
7	0.03-0.07	0.07-0.17	0.13-0.38	0.42-0.87	N/A	N/A
11	0.02-0.07	0.11-0.18	0.10-0.58	0.37-0.46	N/A	N/A
14	0.03-0.05	0.06-0.11	0.21-0.46	0.38-0.56	N/A	N/A
18	0.02-0.04	0.05-0.11	0.17-0.43	0.15-0.29	N/A	N/A
21	0.04-0.07	0.07-0.13	0.08-0.47	0.39-0.51	N/A	N/A
24	0.03-0.05	0.08-0.12	0.11-0.59	0.34-0.80	0.21-0.50	0.37-0.80
28	0.03-0.04	0.12-0.18	0.13-0.47	0.47-0.51	0.45-0.46 [d]	0.30-0.67
31	N/A	N/A	N/A	N/A	0.01-0.02	0.01
35	N/A	N/A	N/A	N/A	N/A	<0.01-0.02
Liver	0.07-0.20	1.18-2.44	2.07-3.47	2.29, 3.80 [d]	0.12-0.67	0.26-0.51
Kidney	1.59-6.48	8.82-18.14	9.70-29.06	23.89, 24.38 [d]	<0.05-0.10	<0.05
Muscle	0.16-0.24	0.28-0.51	0.49-1.13	0.98, 1.02 [d]	0.05-0.06	<0.05
Fat	0.33, 0.51 [e]	0.45-0.75	1.26-3.55	2.03, 2.30 [d]	<0.05-0.12	<0.05

[a] Data are a range of three samples, unless otherwise indicated.

[b] The MTDB for cattle is 881 ppm based on a diet consisting of grass forage (60%), aspirated grain fractions (20%), and milled grain fractions (20%).

[c] N/A = not applicable, or sample not analyzed.

[d] Only two samples were analyzed due to the early sacrifice of one cow.

[e] Data from only two samples are presented as the sample from the third cow was contaminated with other tissue.

Agency Memoranda Cited in this Review

DP Barcode: D216135
Subject: 2,4-D. Protocol for Magnitude of the Residue in Meat/Milk from Inductry Task Force II on 2,4-D Research Data.
From: S. Knizner
To: J. Coombs
Date: 6/15/95
MRID(s): None

DP Barcode: D226556
Subject: 2,4-D. Enforcement Analytical Method for Ruminant and Poultry Commodities.
From: D. Miller
To: P. deschamp
Date: 6/26/96
MRID(s): 44016501 and 44016502

Master Record Identification Number

44024801 Krautter, G., Downs, J. (1996) 2,4-D: Magnitude of the Residues in Meat and Milk of Lactating Dairy Cows: Lab Project Number: 886: 1889: 912. Unpublished study prepared by PTRL East, Inc. 608 p.

2,4-D
Shaughnessy No. 030001; Case 0073
(DP Barcode D235983)

Registrant's Response to Residue Chemistry Data Requirements

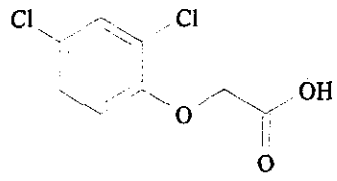
April 6, 1998

Contract No. 68-D4-0010

Submitted to:
U.S. Environmental Protection Agency
Arlington, VA

Submitted by:
Dynamac Corporation
1910 Sedwick Road
Building 100, Suite B
Durham, NC 27713

2,4-D



(Shaughnessy No. 030001. Case No. 0073)

DP Barcode D235983

REGISTRANT'S RESPONSE TO RESIDUE CHEMISTRY DATA REQUIREMENTS

BACKGROUND

The Interregional Research Project No. 4 (IR-4) received minor use Pesticide Clearance Requests from AR, NC, OK, OR and VA Agricultural Experiment Stations for a minor use of 2,4-D amine as a directed spray for the control of weeds in the row middles of blueberries. IR-4 had previously submitted field trial data to support the currently labeled use of 2,4-D in lowbush blueberries. This study was reviewed by the Agency (DP Barcodes D224795 and D224796, D. Miller, 7/17/96) and deemed adequate to support the existing tolerance for 2,4-D *per se* on blueberries (0.1 ppm). In response to the Pesticide Clearance Requests, IR-4 has submitted field studies to support a minor use of 2,4-D in highbush blueberry production systems (1997, MRID 44268501). These data are reviewed here to determine their adequacy in fulfilling residue chemistry data requirements. The Conclusions and Recommendations stated in this review pertain only to the magnitude of the residue in plants.

The nature of the residue in plants is adequately understood based upon acceptable wheat, lemon, and potato metabolism studies. The nature of the residue in livestock is understood based upon acceptable ruminant and poultry metabolism studies. Tolerances for residues of 2,4-D (2,4-dichlorophenoxyacetic acid) in/on plant and processed food/feed commodities are currently expressed in terms of 2,4-D *per se* [40 CFR §180.142(a)(1-6, 9-13) and (b)]. Tolerances in livestock commodities are currently established in terms of residues of 2,4-D and/or its metabolite 2,4-dichlorophenol [40 CFR §180.142(a)(8)]. The HED Metabolism Assessment Review Committee (MARC), on 9/3/03, concluded that the residue of concern in plants and animals is 2,4-D, both free and conjugated, determined as the acid (W. Hazel and L. Taylor, 12/3/03, TXR No. 0052264, D293119). It was also determined that 2,4-dichlorophenol should be removed from the residue definition of 2,4-D tolerances in livestock commodities. A tolerance of 0.1 ppm has been established for residues of 2,4-D in/on blueberries. Adequate

methods are available for data collection. Three GC methods with microcoulometric detection and one GC method with electron capture detection (ECD) are listed in Pesticide Analytical Method (PAM) Vol. II as Methods A, B, C, and D. A new analytical enforcement method for plants has been proposed (DP Barcode D205346, D. Miller, 1/24/96) and independently validated (DP Barcode D216962, D. Miller, 3/19/96).

The Codex and U.S. tolerance expressions for 2,4-D are compatible for plant commodities. Codex MRLs (CXL) for 2,4-D are expressed in terms of parent only and range from 0.05 mg/kg on sorghum and rice to 2.0 mg/kg on citrus. Issues regarding the compatibility of the U.S. tolerances and Codex MRLs will be addressed in the Residue Chemistry Chapter of the 2,4-D RED [W. Hazel, 3/1/04, D287660].

CONCLUSIONS AND RECOMMENDATIONS

1. The GC/ECD method, EN-CAS Method No. ENC-2/93, is adequate for determining residues of 2,4-D in/on highbush blueberries. The method has a validated limit of quantitation (LOQ) of 0.01 ppm.

2. The submitted storage stability data are adequate and indicate that residues of 2,4-D are stable at -20 C for at least 315 days in blueberries. These data adequately support the storage intervals and conditions under which residue samples were held for the current study.

3a. The submitted highbush blueberry data are adequate and indicate that residues of 2,4-D are not likely to exceed the established tolerance of 0.1 ppm in/on blueberries when applied according to the proposed directions for use. The new use pattern supported by the IR-4 study allows for two directed applications of an unspecified 2,4-D amine salt formulation, one after harvest in the late summer or fall and another 30 days prior to harvest at 1.4 lb ae/A/application. In the current study, residues of 2,4-D in/on highbush blueberries harvested following the last of two directed treatments of 2,4-D (1.4 lb ae/A/application) with a PHI of 28-30 days were <0.01-0.013 ppm in/on 12 treated samples.

3b. No blueberry data were provided on residues resulting from use of a 2,4-D ester formulation, which is the only form of 2,4-D currently registered for use on blueberries. Nevertheless, the Agency has previously concluded that 2,4-D residue levels are generally similar on a given crop regardless of the form of 2,4-D used. The available highbush blueberry residue data from the application of an amine salt of 2,4-D will adequately support the use of 2,4-D ester formulations on highbush blueberries.

DETAILED CONSIDERATIONS

Residue Analytical Methods

In conjunction with the magnitude of the residue field study (1997, MRID 44268501), IR-4 submitted a description of a GC/ECD method that is a minor modification of EN-CAS Method No. ENC-2/93 for determining residues of 2,4-D in various plant commodities and processed fractions. Method ENC-2/93 has been reviewed and deemed adequate for determining 2,4-D residues in/on RACs of various crops (DP Barcode D205346, D. Miller, 1/24/96). Sample analyses were performed by the North Dakota IR-4 Satellite Lab, Fargo, ND.

Briefly, residues were extracted with 0.5 M KOH in ethanol:H_2O (1:1, v/v), filtered and then refluxed for 1 hour in 0.22 M HCl. Hydrolyzed residues were then cleaned-up by eluting through a C_{18} solid phase extraction column with hexane:ethyl acetate (1:1, v/v). Residues were then partitioned into 0.1 M Na_2HPO_4, acidified, partitioned into diethyl ether, concentrated to dryness, and then derivatized to the methyl ester using methanolic boron trifluoride. The methylated residues were further cleaned up by treatment with potassium permanganate, partitioned into 35% toluene in trimethylpentane, and cleaned-up using an Alumina column prior to analysis by GC/ECD.

In conjunction with the sample analyses, the registrant submitted method validation and concurrent method recovery data. For method validation, six control samples were fortified with 2,4-D at 0.01 or 0.05 ppm. For concurrent method recovery, eight samples were fortified at 0.009-0.05 ppm of 2,4-D. Overall method recoveries of 2,4-D from fortified control samples were 76-106%. Apparent residues of 2,4-D in 12 control samples of blueberries analyzed along with the fortified and treated samples were <LOQ (0.01 ppm). Adequate sample calculations and example chromatograms were submitted.

The GC/ECD method, EN-CAS Method No. ENC-2/93, is adequate for determining residues of 2,4-D in/on highbush blueberries. The method has a validated LOQ of 0.01 ppm.

Storage Stability Data

In conjunction with the magnitude of the residue study (1997, MRID 44268501), IR-4 has submitted storage stability data on residues of 2,4-D in blueberries. Three blueberry samples were fortified with 2,4-D at 0.05 ppm and held in frozen storage (-20 C) at the analytical laboratory for 315 days . The storage stability samples were analyzed using the adequate GC/ECD method described above. Recoveries of 2,4-D from the stored samples were 93%-100%.

Samples of blueberries from the field studies were stored frozen at ≤0 C for a maximum of 282 days prior to analysis.

The submitted storage stability data are adequate and indicate that residues of 2,4-D are stable at -20 C for at least 315 days in blueberries. These data adequately support the storage intervals and conditions under which residue samples were held for the current study.

Magnitude of the Residue in Plants

A tolerance of 0.1 ppm has been established for residues of 2,4-D in/on blueberries [40 CFR §180.142 (b)].

A REFS search dated 4/1/98 identified four 2,4-D ester EC formulations (EPA Reg. Nos. 62719-9, 62719-50, 42750-20, and 19713-345) registered for use on blueberries. Two of these EPs (EPA Reg. Nos. 62719-50 and 62719-9) are registered for a directed wipe application to weeds growing in lowbush blueberries in ME. Blueberries were not identified as a registered crop in the directions for use under EPA Reg. No. 42750-20, and the label for EPA Reg. No. 19713-345 was not available for review.

IR-4 has submitted data (1997, MRID 44268501) from six tests conducted in MI, NJ, NC (2) and OR (2) depicting residues of 2,4-D in/on highbush blueberries. In one test in each state, 2,4-D (3.8 lb/gal SC/L) formulated as an amine salt was applied twice to blueberries at 1.4 lb ae/A/application, for a total of 2.8 lb ae/A/season (1x the proposed rate). At the OR and NC test sites, 2,4-D was also applied at 2.8 lb ae/A/application (2x). 2,4-D was applied as a spray directed to the row middles after harvest (generally late summer to fall) and again the following growing season 28-31 days prior to harvest. Both applications were made using ground equipment in 20-50 gal water per acre.

Blueberries were harvested at normal crop maturity 28-31 days following the second directed treatment. Four control and four treated samples were collected for each trial at the OR and NC test sites. For the MI and NJ test sites two control and two treated samples were collected from each trial. Samples were frozen within two hours of collection and stored at ≤0 C for 4-24 days prior to shipment by ACDS freezer truck or by overnight delivery on dry ice directly to the IR-4 Satellite Lab, Fargo, ND. Samples were stored at approximately -20 C at the analytical laboratory for 134-251 days prior to analysis. The maximum frozen storage interval from sampling to analysis was 282 days.

Residues were determined using the GC/ECD method described in the Residue Analytical Method section of this report. The LOQ for 2,4-D residues in/on blueberries is 0.01 ppm. Apparent residues of 2,4-D were below the LOQ (<0.01 ppm) in/on 12 control samples. Residues of 2,4-D in/on highbush blueberries harvested following the last of two directed treatments of 2,4-D (1.4 lb ae/A/application) with a PHI of 28-31 days were <0.01-0.013 ppm in/on 12 treated samples (Table 1).

Table 1. Residues of 2,4-D in/on highbush blueberries harvested 28-31 days following the second of two directed spray applications of a 2,4-D amine salt (3.8 lb/gal SC/L) at 1.4 lb ae/A/application.

Test location	Total Application Rate (lb ae/A) [a]	Spray Volume (gal/A)	PTI [b] (days)	2,4-D Residues (ppm)
East Lansing, MI	2.8	20	29	<0.01, 0.011
Castle Hane, NC	2.8	50	28	<0.01, <0.01 <0.01, <0.01
	5.6			<0.01, <0.01 <0.01, <0.01
Bridgeton, NJ	2.8	36	31	<0.01, 0.013
Aurora, OR	2.8	50	30	<0.01, <0.01 <0.01, <0.01
	5.6			<0.01, <0.01 <0.01, <0.01

[a] The proposed 1x rate is 1.4 lb ae/A, applied as a directed spray to the row middles after harvest and again the following growing season ~30 days prior to harvest, for a seasonal application rate of 2.8 lb ae/A.

[b] Days after final application.

Geographic representation is adequate. IR-4 provided data from blueberry growing regions which account for >83% of U.S. blueberry production. A total of six tests were conducted in Region 2 (3 tests), Region 5 (1 test), and Region 12 (2 tests), four tests at 1x and two tests at 2x the proposed use rate.

The submitted highbush blueberry data are adequate and indicate that residues of 2,4-D are not likely to exceed the established tolerance of 0.1 ppm in/on blueberries when applied according to the proposed directions for use. The new use pattern supported by the IR-4 study allows two directed applications of an unspecified 2,4-D amine salt formulation, one after harvest in the late summer or fall and another 30 days prior to harvest at 1.4 lb ae/A/application.

No data were provided on residues resulting from use of a 2,4-D ester formulation, which is the only form of 2,4-D currently registered for use on blueberries. Nevertheless, the Agency has previously reviewed side-by-side studies on a variety of crops comparing 2,4-D residues resulting from the application of the acid, amine salts, and ester formulations of 2,4-D. Because 2,4-D residues are generally similar on a given crop regardless of which of the three forms of 2,4-D is used, the available highbush blueberry residue data from the application of an amine salt will adequately support use of 2,4-D ester formulations on highbush blueberries.

MASTER RECORD IDENTIFICATION NUMBER

44268501 Kunkel, D. (1997) 2,4-D: Magnitude of the Residue on Blueberry (Highbush): Lab Project Number: 3085.93-NDR03: 3085.93-OR18: 3085.93-NC04: 3085.94-NJ16: 3085.94-MI14. Unpublished study prepared by the Interregional Research Project No. 4. 454 p.

AGENCY MEMORANDA CITED IN THIS DOCUMENT

DP Barcode: D224795 and D224796
Subject: Crop Field Trials on Apple, Blueberry, Cranberry, Filbert, Pear, Pecan, Potato, Strawberry, and Sweet Corn and Processing Study on Apples. GDLNs 171-4(k) and 171-4(1).
From: D. Miller
To: P. Deschamps
Date: 7/17/96
MRID(s): 43886401-06, 43943101, 43963801-02

DP Barcode: D205346
Subject: Enforcement Analytical Method for Plants.
From: D. Miller, CB
To: J. Coombs, SRRD
Date: 1/24/96
MRID(s): 43289301

DP Barcode: D216962
Subject: 2,4-D. Independent Method Validation.
From: D. Miller, CB
To: J. Coombs, SRRD
Date: 3/19/96
MRID(s): 43691101

2,4-D
PC Code 030001; Case 0073
(DP Barcode D276792)

Registrant's Response to Residue Chemistry Data Requirements

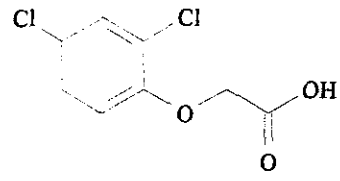
August 11, 2000

Contract No. 68-W-99-053

Submitted to:
U.S. Environmental Protection Agency
Arlington, VA

Submitted by:
Dynamac Corporation
1910 Sedwick Road
Building 100, Suite B
Durham, NC 27713

2,4-D



(PC Code: 030001. Case No. 0073)

DP Barcode D276792

REGISTRANT'S RESPONSE TO RESIDUE CHEMISTRY DATA REQUIREMENTS

BACKGROUND

The Industry Task Force II on 2,4-D Research Data (TF II) submitted data pertaining to 2,4-D residues in fish and shellfish (1996, MRID 44135201 and 1998, MRID 44577801). These data are reviewed here to determine their adequacy in fulfilling residue chemistry data requirements. The Conclusions and Recommendations stated in this review pertain only to the magnitude of the residue in fish and shellfish.

The nature of the residue in animals is understood based upon acceptable ruminant and poultry metabolism studies. The MARC (9/3/03) has concluded that the residues of concern in plants and animals is 2,4-D, free and conjugated, determined as the acid (W. Hazel and L. Taylor, 12/3/03, D293119, TXR No. 0052264). The nature of the residue in fish is adequately understood (DP Barcode D208093, 1/20/95, R. Perfetti). The residue of concern in fish and shellfish is 2,4-D, free and conjugated, determined as the acid.

Tolerances for residues of 2,4-D (2,4-dichlorophenoxyacetic acid) in/on plant and animal commodities are listed at 40 CFR §180.142. Tolerances of 1.0 ppm have been established for residues of 2,4-D *per se* in fish and shellfish [40 CFR §180.142 (a)(6 and 9)]. Tolerances for residues in livestock commodities are currently established in terms of residues of 2,4-D and/or its metabolite 2,4-dichlorophenol and range from 0.1 ppm to 2.0 ppm [40 CFR §180.142 (a)(8)]. Adequate methods are available for data collection from animal tissues. A proposed enforcement method for animal commodities is adequate pending submission of confirmatory data and Agency validation (DP Barcode D226556, 6/26/96, D. Miller).

CONCLUSIONS AND RECOMMENDATIONS

1. The GC/ECD (or MSD) method used in this study is essentially the same as that proposed for

enforcement of tolerances for 2,4-D residues in ruminant and poultry commodities, and is adequate for data collection from fish and shellfish. This method is adequate for tolerance enforcement, contingent upon receipt of additional information required by HED (DP Barcode D226556, 6/26/96, D. Miller).

2a. The Task Force II submitted two studies pertaining to fish and shellfish exposed to 2,4-D at 6.0 ppm in a static aquatic test system. The report stated that 6.0 ppm is the maximum expected environmental concentration of 2,4-D from registered uses; HED concurs. The 3.8 lb ae/gal SC formulation of dimethyl amine and triisopropylamine salts may be applied at a maximum rate of 38 lb ae/A.

2b. In one study conducted in 1996, catfish, bluegill, and crayfish were exposed to 2,4-D for 15 days. Maximum 2,4-D residues in catfish and bluegill were 0.054-0.070 ppm, reached after 6 hours of exposure. In crayfish, maximum residues of 1.1 ppm were attained on day 8.

2c. In the second study, clams and crayfish were exposed to 2,4-D for 28 days. Maximum residues in clams were 0.59 ppm after 2 days and maximum residues in crayfish were 1.1 ppm after 14 days.

2d. These studies on fish and shellfish are adequate. HED concludes that these data support the established tolerance of 1.0 ppm for 2,4-D residues in shellfish; the tolerance for 2,4-D residues in fish can be reduced to 0.1 ppm.

DETAILED CONSIDERATIONS

OPPTS GLN 860.1340: Residue Analytical Methods

GC methods were used to collect data on water, fish and shellfish in the two submitted studies. For analyses of water, residues were extracted with methyl-*tert*-butyl ether (MTBE) following addition of H_3PO_4. Isooctane was added and the extract concentrated. Residues were methylated with BF_3/MeOH and analyzed by GC/ECD.

Residues in fish and shellfish were analyzed using a method very similar to that proposed as an enforcement method for ruminant tissues. Samples are refluxed in 2N HCl. The aqueous hydrolyzed extract is diluted with ACN and is subsequently cleaned up on a Florisil column to remove matrix interferences. The resultant extract is diluted with 1% NaOH and acidified following rotary evaporation, and residues are partitioned into a solution of 10% EtOAc in hexane. The organic phase is passed through a neutral alumina column and the analyte eluted with a solution of MeOH in NaOH. The extract is acidified and partitioned with MTBE, the MTBE layer is concentrated and the residues are methylated with BF_3/MeOH. Derivatized residues are analyzed by GC/ECD (fish and crayfish) or GC/MSD (clam). The limit of quantitation was 0.01 ppm. Recoveries in the 1996 study were 71.4-84% (n=5) from catfish,

74.8-114% (n=6) from bluegill, 82.8-118.2% (n=5) from crayfish samples fortified at 0.01 or 0.05 ppm, and 83.0-119% (n=15) from water fortified at 0.1 or 6.0 ppm. In the 1998 study, recoveries were 108 and 119% from two samples of clams fortified at 0.2 ppm, 74.2-93.5% (n=7) from crayfish fortified at 0.10 ppm, and 101-117% (n=7) from water fortified at 6.0 ppm. Apparent residues in untreated edible tissues from fish, clams, and crayfish were <0.001-0.012 ppm (1996) or 0.002-0.004 ppm (1998).

The GC/ECD (or MSD) method used in this study is essentially the same as that proposed for enforcement of 2,4-D tolerances in ruminant and poultry commodities and is adequate for data collection on fish and shellfish. This method is adequate for tolerance enforcement, contingent upon receipt of additional information required by HED (DP Barcode D226556, 6/26/96, D. Miller).

OPPTS GLN 860.1400: Magnitude of the Residue in Fish and Shellfish

A tolerance of 1.0 ppm has been established for residues of 2,4-D in fish and shellfish [40 CFR §180.142 (a)(6 and9)].

The Industry Task Force II (1996, MRID 44135201 and 1998, MRID 44577801) submitted two studies pertaining to residues of 2,4-D in fish and shellfish resulting from exposure to 2,4-D in water in a static system. 2,4-D was applied as the DMA salt to water at a nominal concentration of 6.0 mg ae/L, the maximum expected environmental concentration of 2,4-D in water.

The in-life phases of the studies were conducted by Springhorn Laboratories, Inc., Health and Environmental Sciences, Wareham, MA. Each test employed seven replicate fiberglass cylindrical 1,000 L treatment vessels and one control vessel containing the dilution water and a uniform layer of sandy soil. In Test 1, each pool contained 8 channel catfish, 7 bluegill sunfish, 19 crayfish and 6 clams (data on clams were not reported for Test 1, owing to method recovery problems). The "Amine 400" 2,4-D product was added to the treatment pools at a target concentration of 6 mg ae/L. Water and organisms were sampled at 3, 6, and 12 hours after addition of 2,4-D and on days 1, 2, 8, and 16. In Test 2, 29 crayfish and 19 clams were added to each pool and allowed to acclimate for 2 days prior to addition of 2,4-D. Water, clams and crayfish were sampled at 2 hours, 12 hours, and on days 2, 7, 14, 21, and 28. Water hardness, pH, dissolved oxygen, and 2,4-D concentrations were monitored throughout each test. Samples were frozen and stored for up to 1 year prior to analysis.

The analytical phase was conducted by PTRL, East, Inc., Richmond, KY. Residues were analyzed using the method described above. The results are summarized in Tables 1 and 2.

Table 1. Residues of 2,4-D in edible tissue from channel catfish, bluegill, and crayfish exposed in static systems dosed at a nominal concentration of 6.0 mg ae/L (Test 1).

Interval	Residues (ppm) [a]		
	Catfish	Bluegill	Crayfish
3 hr	0.045, 0.039	0.043, 0.045	0.12, 0.061
6 hr	0.063, 0.070	0.067, 0.054	0.072, 0.060
12 hr	0.056, 0.049	0.055, 0.029	0.23, 0.077
1 day	0.036, 0.058	0.048, 0.052	0.47
2 day	0.027, 0.051	0.028, 0.042	0.35
8 day	0.020	0.012, 0.010	1.1
15 day	NA	0.017, 0.013	1.0

[a] Data are from one fish per interval, one or two crayfish per interval.

Table 2. Residues of 2,4-D in edible tissue from clams and crayfish exposed in static systems dosed at a nominal concentration of 6.0 mg ae/L (Test 2).

Interval	Residues (ppm)	
	Clams	Crayfish
2 hr	0.29	0.08, 0.07 [a]
12 hr	0.52	0.10
2 day	0.59	0.21, 0.19
7 day	0.41	0.82, 0.65
14 day	0.19	1.18, 1.01[b]
21 day	0.02	0.59, 0.52[b]
28 day	<0.01	0.73, 0.61[b]

[a] Duplicate samples

[b] Repeat analysis of the same sample.

Conclusions. Following exposure to 2,4-D at 6.0 ppm in a static system, maximum 2,4-D residues in catfish and bluegill were 0.067-0.070 ppm, reached after 6 hours of exposure. In crayfish from Test 1, maximum residues of 1.1 ppm were attained on day 8. In the second study, maximum residues in clams were 0.59 ppm after 2 days and maximum residues in crayfish were 1.1 ppm after 14 days. These data support the established tolerance of 1.0 ppm for 2,4-D residues in shellfish; the data indicate that the tolerance for residues in fish can be reduced to 0.1 ppm.

Agency Memoranda Cited in this Review

DP Barcode: D208093
Subject: 2,4-D. Response to the 2,4-D Registration Standard: Fish Metabolism Study.
From: R. Perfetti
To: E. Saito
Date: 1/20/95
MRID(s): 43378801

DP Barcode: D226556
Subject: 2,4-D. Enforcement Analytical Method for Ruminant and Poultry Commodities.
From: D. Miller
To: P. Deschamp
Date: 6/26/96
MRID(s): 44016501 and 44016502.

Master Record Identification Numbers

44135201 Biever, R. C. (1996) A Freshwater Fish and Shellfish Magnitude of Residues Study in a Static Aquatic System: Amine 400 2,4-D Weed Killer: Lab Project Number: 3140.0796.6106.395:96-9-6660: 1064. Unpublished study prepared by Springhorn Labs, Inc. and PTRL East, Inc. 167 p.

44577801 Biever, R. C. (1998) A Freshwater Shellfish Magnitude of Residues Study in a Static Aquatic System with 2,4-D Dimethylamine salt: Lab Project Number: 3140.0796.6107.395:1081. Unpublished study prepared by Springhorn Labs, Inc. and PTRL East, Inc. 133 p.

2,4-D
PC Code 030001; Case 0073
(DP Barcode D276792)

Registrant's Response to Residue Chemistry Data Requirements

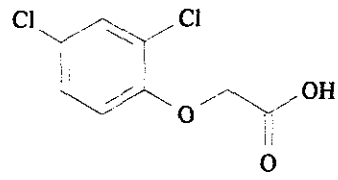
August 11, 2000

Contract No. 68-W-99-053

Submitted to:
U.S. Environmental Protection Agency
Arlington, VA

Submitted by:
Dynamac Corporation
1910 Sedwick Road
Building 100, Suite B
Durham, NC 27713

2,4-D



(PC Code: 030001. Case No. 0073)

DP Barcode D276792

REGISTRANT'S RESPONSE TO RESIDUE CHEMISTRY DATA REQUIREMENTS

BACKGROUND

In response to outstanding data requirements for reregistration of 2,4-D, the Industry Task Force II on 2,4-D Research Data (TF II) submitted data pertaining to residues in/on almonds (1996, MRID 44211901). These data are intended to support the established tolerance for 2,4-D residues in "nuts."

In addition, the TF II submitted data on wheat raw agricultural commodities (1996, MRIDs 44190301 and 44190302) following treatment with the 2,4-D dimethylamine salt (DMA) or the 2-ethylhexyl ester (2-EHE) following applications totaling 1.75 lb ae/A. Previously, data reflecting the same use pattern were reviewed (DP Barcode D220447, 4/5/96, D. Miller); eight tests were conducted in CA(2), GA, KS, MN, ND, OK, and WA. The review concluded that tolerance revisions would be required pending label revisions specifying rates and PHIs reflected in the study. The present data on wheat are from studies conducted in CO, GA, ND(2), OK, and WA. These data are reviewed here to determine their adequacy in fulfilling residue chemistry data requirements. The Conclusions and Recommendations stated in this review pertain only to the magnitude of the residue in plants.

The nature of the residue in plants is adequately understood based upon acceptable wheat, lemon, and potato metabolism studies. The nature of the residue in animals is understood based upon acceptable ruminant and poultry metabolism studies. The HED Metabolism Committee (6/16/93) determined that there is no need to require data for 2-ethylhexanol in crops treated with the 2-ethylhexyl ester of 2,4-D. The HED Metabolism Assessment Review Committee (MARC), on 9/3/03, concluded that the residue of concern in plants and animals is 2,4-D, both free and conjugated, determined as the acid (W. Hazel and L. Taylor, 12/3/03, TXR No. 0052264,

D293119). It was also determined that 2,4-dichlorophenol should be removed from the residue definition of 2,4-D tolerances in livestock commodities.

Tolerances for residues of 2,4-D (2,4-dichlorophenoxyacetic acid) in/on plant and processed food/feed commodities are expressed in terms of 2,4-D *per se* [40 CFR §180.142 (a)(1-6, 8-13) and (b)]. A tolerance of 0.1 ppm has been established for residues of 2,4-D in "nuts," 0.5 ppm in wheat grain, and 20 ppm in/on several grain forages [40 CFR §180.142 (a)(2)]. Tolerances for residues in animal commodities are currently established in terms of residues of 2,4-D and/or its metabolite 2,4-dichlorophenol [40 CFR §180.142 (a)(8)]. Adequate methods are available for data collection. Three GC methods with microcoulometric detection and one GC method with electron capture detection (ECD) are listed in Pesticide Analytical Method (PAM) Vol. II as Methods A, B, C, and D. A new analytical enforcement method for plants has been proposed (DP Barcode D205346, D. Miller, 1/24/96) and independently validated (DP Barcode D216962, D. Miller, 3/19/96).

The Codex and U.S. tolerance expression for 2,4-D are compatible for plant commodities. Codex MRLs (CXL) for 2,4-D are expressed in terms of parent only and range from 0.05 mg/kg on sorghum and rice to 2.0 mg/kg on citrus. Issues regarding the compatibility of the U.S. tolerances and Codex MRLs will be addressed when the reregistration eligibility decision for 2,4-D is made.

CONCLUSIONS AND RECOMMENDATIONS

1. The GC/ECD method, EN-CAS Method No. ENC-2/93, is adequate for determining residues of 2,4-D in/on almond and wheat commodities. The method has validated limits of quantitation (LOQ) of 0.01 ppm for wheat commodities and 0.05 ppm for almond matrices.

2a. Storage stability data were submitted with the almond trials. Untreated samples of nutmeats and hulls were fortified with 2,4-D at 0.50 ppm and stored frozen for 48 days. Recoveries were 69-73% from three nutmeat samples and 80-96% from three hull samples. In addition, the registrants cited data from a previous study indicating 80-89% recovery from pecan nutmeats fortified with 2,4-D at 0.50 ppm and stored for 182 days. These data support the current almond residue trials.

2b. Previously submitted storage stability data are adequate and indicate that residues of 2,4-D are stable in wheat at -20 C for the storage intervals and conditions under which residue samples were held for the current study.

Almond

3a. Ten tests were conducted in CA reflecting two applications to almond orchards of the 2,4-D dimethylamine salt (DMA) 3.8 lb ae/gal SC or 2-EHE 3.8 lb ae/gal EC formulation at a target rate of 1.425 lb ae/A.

3b. 2,4-D residues were <0.05 ppm (<LOQ) - 0.160 ppm in almond nutmeats and <0.05-0.098 ppm in/on almond hulls. These data support the established tolerance of 0.2 ppm for 2,4-D residues in/on nuts. A tolerance of 0.1 ppm would be appropriate for 2,4-D residues in/on almond hulls. The 40 CFR listing for "nuts" should be revised to "Tree Nuts Group."

Wheat

4a. Six tests each were conducted with wheat using either the 2,4-D DMA salt or the 2-EHE form, applied at 1.25 lb ae/A at tillering followed by a pre-harvest application at 0.5 lb ae/A. Including previous studies, a total of 14 tests were conducted with each 2,4-D form. Wheat forage was collected 7 and 14 days after the early treatment. Wheat grain and straw were harvested 7 and 14 days after the second application.

A previous study (1995) covering eight tests with each form of 2,4-D reflecting this same use pattern has been reviewed by HED (DP Barcode D220447, 4/5/96, D. Miller); eight tests were conducted in CA(2), GA, KS, MN, ND, OK, and WA. In the previous tests the following maximum residues were obtained for respective PHIs of 7 and 14 days: (i) 56.7 or 24.9 ppm in forage; (ii) 1.86 or 1.39 ppm in grain; and (iii) 23.7 or 40.9 ppm in/on straw.

Overall, the residues detected in the current (1996) tests were of the same magnitude as those from the previous study. Together, the current and previous submissions adequately cover the geographical areas specified in the Guidance Document.

4b. *Forage.* Maximum 2,4-D residues were 56.2 and 22.5 ppm in/on forage harvested 7 and 14 days following a single application at tillering at 1.25 lb ae/A; previous studies showed maximum residues of 56.7 and 24.9 ppm. Provided that all labels are revised to specify an at-tillering rate of 1.25 lb ae/A, **the established tolerance for 2,4-D residues in wheat forage should be increased to 60 ppm for a 7-day PHI/pregrazing interval (PGI) or 30 ppm for a 14-day PHI/PGI.**

4c. *Grain.* Maximum 2,4-D residues were 0.418 ppm at a 7-day PHI and 0.226 ppm at a 14-day PHI following total seasonal application at 1.75 lb ae/A. Previous studies showed maximum residues of 1.86 and 1.39 ppm at 7 and 14 days after treatment, respectively. Provided that all labels are revised to specify the use pattern reflected in these studies, **the established tolerance for 2,4-D residues in wheat grain should be increased to 2.0 ppm** for either a 7- or 14-day PHI.

4d. *Straw.* Maximum 2,4-D residues were 13.5 ppm at a 7-day PHI and 17.1 ppm at a 14-day PHI following total seasonal application at 1.75 lb ae/A. Previous studies showed maximum residues of 23.7 and 40.9 ppm at 7 and 14 days after treatment, respectively. Provided that all labels are revised to specify the use pattern reflected in these studies, **the established tolerance for 2,4-D residues in wheat straw should be increased to 50 ppm** for either a 7- or 14-day PHI.

4e. No data were submitted for wheat hay. These data are required.

4f. The 2,4-D residue data on wheat commodities will be used to support tolerances for 2,4-D residues in/on the corresponding commodities of barley, millet, oats, and rye.

DETAILED CONSIDERATIONS

GLN 860.1360: Residue Analytical Methods

In conjunction with the magnitude of the residue field studies on almonds and wheat, Task Force II submitted descriptions of a GC/ECD method that is a minor modification of EN-CAS Method No. ENC-2/93 for determining residues of 2,4-D in various plant commodities and processed fractions. Method ENC-2/93 has been reviewed and deemed adequate for determining 2,4-D residues in/on RACs of various crops (DP Barcode D205346, D. Miller, 1/24/96). Sample analyses for the current studies were performed by Corning Hazleton, Inc. (CHW), Madison, WI.

Briefly, residues are extracted with 0.5 M KOH in ethanol:H_2O (1:1, v/v), filtered and then refluxed for 1 hour in 0.22 M HCl. Hydrolyzed residues are then cleaned-up by eluting through a C_{18} solid phase extraction column with hexane:ethyl acetate (1:1, v/v). Residues are partitioned into 0.1 M Na_2HPO_4, acidified, partitioned into diethyl ether, concentrated to dryness, and derivatized to the methyl ester using methanolic boron trifluoride. The methylated residues are further cleaned up by treatment with potassium permanganate, partitioned into 35% toluene in trimethylpentane, and cleaned-up using an Alumina column prior to analysis by GC/ECD.

Recoveries determined concurrently with the almond field residue samples were 72-97% and 67-103%, respectively, from almond nutmeats and hulls fortified at 0.05-0.5 ppm. In conjunction with the wheat sample analyses, the registrant submitted concurrent method recovery data (Table 2). For concurrent method recovery, 10-14 samples each of forage, grain, and straw were fortified with 2,4-D at 0.100-60 ppm, 0.010-1.00 ppm, and 0.010-20.0 ppm, respectively. Concurrent recoveries (Table 1) were 60.4-120% from forage, 52.6-130% from grain, and 60.0-120% from straw. For the DMA salt trials, apparent residues of 2,4-D in control samples were <0.01-0.040 ppm in 12 forage samples, <0.01 ppm in six grain samples, and <0.01-0.036 ppm in six straw samples. For the 2-EHE trials, apparent residues of 2,4-D in control samples were <0.01-0.028 ppm in 12 forage samples, <0.01-0.01 ppm in six grain samples, and <0.01-0.030 ppm in six straw samples. Adequate sample calculations and example chromatograms were submitted.

The GC/ECD method, EN-CAS Method No. ENC-2/93, is adequate for determining residues of 2,4-D in/on almond nutmeats and hulls and wheat forage, grain, and straw. The method has a validated LOQ of 0.01 ppm.

Table 1. Recovery of 2,4-D from fortified almond nutmeat and hull samples using method EN-CAS 2/93.

Sample	Fortification (ppm)	Recovery (%)
Nutmeats	0.050	72-97 (n=5), mean 89
	0.50	75-88 (n=8), mean 81
Hulls	0.050	67-103 (n=7), mean 91
	0.50	79-99 (n=8), mean 87

Table 2. Concurrent recoveries of 2,4-D from fortified wheat samples using method EN-CAS 2/93.

Samples	Fortification (ppm)	DMA Recovery (%)	2-EHE Recovery (%)
Wheat forage	0.010	89.8, 110	120
	0.100	74.0, 82.0, 83.0, 91.0	74.0, 84.0, 84.0, 92.0, 94.0, 116
	1.00	81.3, 84.4	103
	5.00	60.4, 93.6	87.3
	10.0	99.1	89.7
	30.0	70.7	N/A
	60.0	N/A	87.3
Wheat grain	0.010	110, 130	91.0
	0.050	72.0, 88.0	78.0
	0.100	74.0, 86.0	102
	0.200	91.0, 84.0	85.0
	0.500	76.0, 52.6	61.6, 62.8, 70.8, 76.2, 90.2
	1.00	62.1	60.8, 63.1, 68.3, 68.4, 78.4
Wheat Straw	0.010	110, 110	88.5
	0.020	N/A	120
	0.100	60.0	109
	0.200	82.5	81.0, 87.5
	0.500	89.2	70.4, 73.8, 79.0
	1.00	70.4, 60.1, 67.4, 76.5	70.4, 95.4
	5.00	88.5	70.4
	10.0	62.8	90.1

	20.0	80.3	71.9

GLN 860.1380: Storage Stability Data

New storage stability data were submitted with the almond field trials (1997, MRID 44211901). Untreated samples of nutmeats and hulls were fortified with 2,4-D at 0.50 ppm and stored frozen for 48 days. Recoveries were 69-73% from three nutmeat samples and 80-96% from three hull samples. In addition, the registrants cited data from a previous study indicating 80-89% recovery from pecan nutmeats fortified with 2,4-D at 0.50 ppm and stored for 182 days. These data support the current almond residue trials.

GLN 860.1500: Magnitude of the Residue in Plants

Almonds

A tolerance of 0.2 ppm is established for residues of 2,4-D in/on nuts [40 CFR 180.142 (b)]. No tolerance has been established for 2,4-D residues in/on almond hulls.

IR-4 submitted data (1997, MRID 44211901) from five tests conducted in CA depicting 2,4-D residues in/on almond nutmeats and almond hulls following orchard floor treatment using the 2,4-D dimethylamine salt (DMA) 3.8 lb ae/gal SC or 2-EHE 3.8 lb ae/gal EC formulation at a target rate of 1.425 lb ae/A. Actual rates of 1.64 lb ae/A of the DMA formulation were applied at two locations. A second application was made 30 days after the first and almonds were harvested 56 days later. The samples were stored for a total of 56 days prior to analysis using the GC/ECD method described above. Recoveries were adequate and residues were <LOQ (0.05 ppm) in/on 10 control samples each of nutmeats and hulls.

Conclusions. The results of the almond field trials are summarized in Table 3. 2,4-D residues were <0.05 ppm (<LOQ) in/on nine samples from treatment with the 2-EHE and eight samples treated with the DMA salt. One sample from a 2-EHE test contained residues of 0.077 ppm and two DMA samples contained 0.112 and 0.160 ppm of 2,4-D residue. Residues in/on almond hulls were <0.05-0.098 ppm. These data support the established tolerance of 0.2 ppm for 2,4-D residues in/on nuts. A tolerance of 0.1 ppm would be appropriate for 2,4-D residues in/on almond hulls. The 40 CFR listing for "nuts" should be revised to "Tree Nuts Group."

Table 3. Residues of 2,4-D in almonds and almond hulls harvested 57 days following two applications.

Trial	Rate (lb ae/A)	Formulation	Residues (ppm)
Almond nutmeats			
CA-16	1.425	DMA	<0.05, <0.05
	1.425	2-EHE	<0.05, <0.05
CA-17	1.425	DMA	<0.05, <0.05
	1.425	2-EHE	<0.05, <0.05
CA-18	1.425	DMA	<0.05, <0.05
	1.425	2-EHE	<0.05, <0.05
CA-19	1.64	DMA	0.112, 0.160 (0.123-0.234)
	1.46	2-EHE	<0.05, 0.077
CA-20	1.425	DMA	<0.05, <0.05
	1.425	2-EHE	<0.05, <0.05
Almond hulls			
CA-16	1.425	DMA	<0.05, <0.05
	1.425	2-EHE	<0.05, <0.05
CA-17	1.425	DMA	<0.05, <0.05
	1.425	2-EHE	<0.05, 0.061
CA-18	1.425	DMA	<0.05, <0.05
	1.425	2-EHE	<0.05, <0.05
CA-19	1.64	DMA	0.098, 0.050
	1.46	2-EHE	<0.05, 0.07
CA-20	1.425	DMA	<0.05, <0.05
	1.425	2-EHE	<0.05, <0.05

Wheat

Tolerances of 0.5 and 20 ppm have been established for residues of 2,4-D in/on wheat grain and forage, respectively [40 CFR §180.142(a)(2)]. Tolerances are not established for 2,4-D in/on wheat hay or straw.

TF II submitted data from field trials conducted at six locations, CO, GA, ND (2), OK, and WA, pertaining to 2,4-D residues in wheat following application of 2,4-D DMA (MRID 44190302). Forage was collected 7 or 14 days after treatment (DAT) and grain and straw were collected 69-105 DAT following one application of the Amine 400 (DMA salt SC/L formulation), at tillering, at 1.25 lb ae/A. In addition, grain and straw were harvested after application at 1.25 lb ae/A at tillering followed by application at 0.5 lb ae/A, 7 or 14 days prior to harvest. Duplicate samples were collected from each test. Samples were shipped frozen to CHW. Forage, grain, and straw samples were held in frozen storage for intervals up to 99 days for forage, 109 days for grain, and 91 days for straw.

In addition, tests reflecting the same use were conducted in the same location with the 2,4-D 2-EHE (MRID 44190301). Forage, grain, and straw were harvested following one application of the (2-EHE EC formulation), at tillering, at 1.25 lb ae/A. In addition, grain and straw were harvested after application at 1.25 lb ae/A at tillering followed by application at 0.5 lb ae/A, 7 or 14 days prior to harvest. Duplicate samples were collected from each test. Samples were shipped frozen to CHW. Forage, grain, and straw samples were held in frozen storage for intervals up to 106 days for forage, 127 days for grain, and 91 days for straw.

2,4-D residue analyses were conducted using the GC/ECD method described above. The method is adequate for data collection. The results of residue analysis are presented in Tables 2-4.

Table 4. Residues of 2,4-D in **wheat forage** following application of the DMA salt or 2-EHE at tillering at 1.25 lb ae/A.

Trial location	PHI	Residues (ppm) DMA salt	Residues (ppm) 2-EHE
CO	7	8.77, 9.13	29.1, 28.2
	14	3.61, 3.77	17.1, 19.1
GA	7	23.6, 17.7	39.7, 26.4
	14	18.2, 11.6	21.0, 20.4
ND1	7	13.6, 14.2	22.9, 21.7
	14	7.66, 7.50	2.57, 3.17
ND2	7	6.30, 4.59	13.7, 14.0
	14	0.651, 0.586	1.34, 1.72
OK	7	25.1, 24.6	56.2, 54.2
	14	6.13, 9.38	22.5, 19.5
WA	7	13.6, 15.1	35.3, 30.0
	14	7.42, 8.03	17.6, 15.1

Table 5. Residues of 2,4-D in **wheat grain** following application of the DMA salt or 2-EHE.

Trial location	Rate (lb ae/A)	PHI	Residues (ppm) DMA	Residues (ppm) 2-EHE
CO	1.25 + 0.5 [a]	7	0.747, 0.896	0.418, 0.414
		14	0.246, 0.011	0.212, 0.213
	1.25 [b]	98	<0.01, <0.01	0.16, 0.29
GA	1.25 + 0.5	7	0.164, 0.143	0.075, 0.051
		14	0.344, 0.270	0.094, 0.127
	1.25	73-75	<0.01, <0.01	<0.01, 0.017
ND1	1.25 + 0.5	7	0.358, 0.248	0.193, 0.177
		14	0.167, 0.145	0.201, 0.226
	1.25	69	<0.01, <0.01	<0.01, <0.01
ND2	1.25 + 0.5	7	0.567, 0.565	0.391, 0.030
		14	0.443, 0.458	0.224, 0.205
	1.25	68-69	<0.01, <0.01	<0.01, <0.01
OK	1.25 + 0.5	7	0.082, 0.028	0.085, 0.075
		14	0.097, 0.241	0.160, 0.138
	1.25	84	<0.01, <0.01	<0.01, 0.032
WA	1.25 + 0.5	7	0.219, 0.115	0.162, 0.171
		14	0.084, 0.163	0.156, 0.168
	1.25	105	<0.01, <0.01	<0.01, <0.01

[a] Application at 1.25 lb ae/A at tillering followed by application at 0.5 lb ae/A prior to harvest.
[b] Application at 1.25 lb ae/A at tillering

Table 6. Residues of 2,4-D in **wheat straw** following application of the DMA salt or 2-EHE

Trial location	Rate (lb ae/A)	PHI	Residues (ppm) DMA	Residues (ppm) 2-EHE
CO	1.25 + 0.5 [a]	7	23.1, 14.0	12.7, 11.9
		14	3.38, 3.93	4.35, 3.88
	1.25 [b]	98	3.46, 0.098	0.112, 0.071
GA	1.25 + 0.5	7	20.5, 15.2	10.4, 12.6
		14	14.7, 10.5	8.76, 11.5
	1.25	73-75	0.521, 0.575	0.463, 0.278
ND1	1.25 + 0.5	7	14.8, 10.0	7.19, 2.70
		14	3.61, 2.58	3.08, 2.67
	1.25	69	0.011, <0.01	<0.01, <0.01
ND2	1.25 + 0.5	7	21.4, 22.9	13.5, 10.5
		14	6.15, 5.40	6.87, 6.37
	1.25	68-69	<0.01, <0.01	0.011, 0.012
OK	1.25 + 0.5	7	2.47, 2.05	4.77, 4.58
		14	7.14, 5.10	5.19, 4.69
	1.25	84	0.041, 0.056	0.043, 0.039
WA	1.25 + 0.5	7	2.39, 2.34	6.30, 1.74
		14	6.78, 6.13	16.7, 17.1
	1.25	105	<0.01, 0.225	0.049, 0.049

[a] Application at 1.25 lb ae/A at tillering followed by application at 0.5 lb ae/A prior to harvest.
[b] Application at 1.25 lb ae/A at tillering

Residues in forage harvested 7 and 14 days, respectively, after DMA salt treatment at tillering were 4.59-25.1 ppm and 0.586-18.2 ppm; after treatment with the 2-EHE, respective residues in forage were 13.7-56.2 ppm and 1.34-22.5 ppm.

Residues in grain harvested 7 and 14 days, respectively, after two DMA salt treatments were 0.028-0.896 ppm and 0.011-0.458 ppm; residues after 2-EHE treatment were 0.030-0.418 ppm and 0.094-0.226 ppm. Residues in grain at harvest (68-105 days) after a single application of either the salt or ester at tillering were <0.01-0.29 ppm.

Residues in straw harvested 7 and 14 days, respectively, after two DMA salt treatments were 2.05-23.1 ppm and 2.58-14.7 ppm; residues after 2-EHE treatment were 1.74-13.5 ppm and

2.67-17.1 ppm. Residues in straw at harvest (68-105 days) after a single application of either the salt or ester at tillering were <0.01-3.46 ppm.

Storage Stability

Total storage times were 29-99 days for forage, 44-109 days for grain, and 14-91 days for straw. Data reviewed previously demonstrated that 2,4-D is stable in wheat forage, grain, and straw stored frozen for 12 months (DP Barcode D220451, 3/19/96, D. Miller).

Conclusions

Six tests each were conducted with wheat using either the 2,4-D DMA salt or the 2-EHE form, applied at 1.25 lb ae/A at tillering followed by a pre-harvest application at 0.5 lb ae/A. A previous study (1995) covering eight tests with each form of 2,4-D reflecting this same use pattern has been reviewed by HED (DP Barcode D220447, 4/5/96, D. Miller); eight tests were conducted in CA(2), GA, KS, MN, ND, OK, and WA. In the previous tests the following maximum residues were obtained at respective PHIs of 7 and 14 days: (i) 56.7 or 24.9 ppm in forage; (ii) 1.86 or 1.39 ppm in grain; and (iii) 23.7 or 40.9 ppm in/on straw. Including previous studies, a total of 14 tests each were conducted with the 2-EHE and DMA salt forms of 2,4-D.

Forage. Maximum 2,4-D residues were 56.2 and 22.5 ppm in/on forage harvested 7 and 14 days following a single application at tillering at 1.25 lb ae/A; previous studies showed maximum residues of 56.7 and 21.0 ppm. Provided that all labels are revised to specify an at-tillering rate of 1.25 lb ae/A, the established tolerance for 2,4-D residues in forage should be increased to 60 ppm for a 7-day PHI/pregrazing interval (PGI) or 30 ppm for a 14-day PHI/PGI.

Grain. Maximum 2,4-D residues were 0.418 ppm at a 7-day PHI and 0.226 ppm at a 14-day PHI following total seasonal application at 1.75 lb ae/A. Previous studies showed maximum residues of 1.86 and 1.39 ppm at 7 and 14 days after treatment, respectively. Provided that all labels are revised to specify the use pattern reflected in these studies, the established tolerance for 2,4-D residues in wheat grain should be increased to 2.0 ppm for either a 7- or 14-day PHI.

Straw. Maximum 2,4-D residues in straw were 13.5 ppm at a 7-day PHI and 17.1 ppm at a 14-day PHI following total seasonal application at 1.75 lb ae/A. Previous studies showed maximum residues of 23.7 and 40.9 ppm at 7 and 14 days after treatment, respectively. Provided that all labels are revised to specify the use pattern reflected in these studies, the established tolerance for 2,4-D residues in wheat straw should be increased to 50 ppm for either a 7- or 14-day PHI.

MASTER RECORD IDENTIFICATION NUMBERS

44190301 Carringer, S. (1996) Magnitude of the Residue of 2,4-D Acid in Wheat (Winter and Spring) Following Ground Applications with 2,4-D 2-Ethylhexyl Ester: (Final Report): Lab Project Number: AA960501: CHW 6397-164. Unpublished study prepared by American Agricultural Services, Inc. and Corning Hazleton, Inc. 498 p.

44190302 Carringer, S. (1996) Magnitude of the Residue of 2,4-D Acid in Wheat (Winter and Spring) Following Ground Applications with 2,4-D Dimethylamine Salt: (Final Report): Lab Project Number: AA960502: CHW 6397-163. Unpublished study prepared by American Agricultural Services, Inc. and Corning Hazleton, Inc. 498 p.

44211901 Cancel, D. (1997) 2,4-D: Magnitude of the Residue in Almond: (Draft Report): Lab Project Number: 4306.96-CAR08: 4306.96-CA16: 4306.96-CA17. Unpublished study prepared by Interregional Research Project No. 4. 539 p.

AGENCY MEMORANDA CITED IN THIS DOCUMENT

DP Barcode: D220451
Subject: 2,4-D. Storage Stability Study on Various Raw and Processed Agricultural Commodities.
From: D. Miller, CBII
To: J. Coombs, SRRD
Date: 3/19/96
MRID(s): 43809901

DP Barcode: D220447
Subject: 2,4-D. Magnitude of the Residue in Wheat.
From: D. Miller, CBII
To: J. Coombs, SRRD
Date: 4/5/96
MRID(s): 43797901 and 43797903

13544

R103131

Chemical:	**2-4,D**
PC Code:	**030001**
HED File Code	***11000 Chemistry Reviews***
Memo Date:	**03/01/2004**
File ID:	**DPD235983; DPD276792; DPD283959; DPD285505**
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